Journal of
DAIRY SCIENCE

Vol. 41 October, 1958 Number 10

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TWENTY-FIRST DAIRY INDUSTRIES EXPOSITION

Navy Pier, Chicago, Illinois

December 8–13, 1958

Participating groups: Milk Industry Foundation, International Association of Ice Cream Manufacturers, American Butter and Cheese Institutes, Evaporated Milk Association, Dairy Society International, National Ice Cream Mix Association, and Dairy Suppliers Foundation.

JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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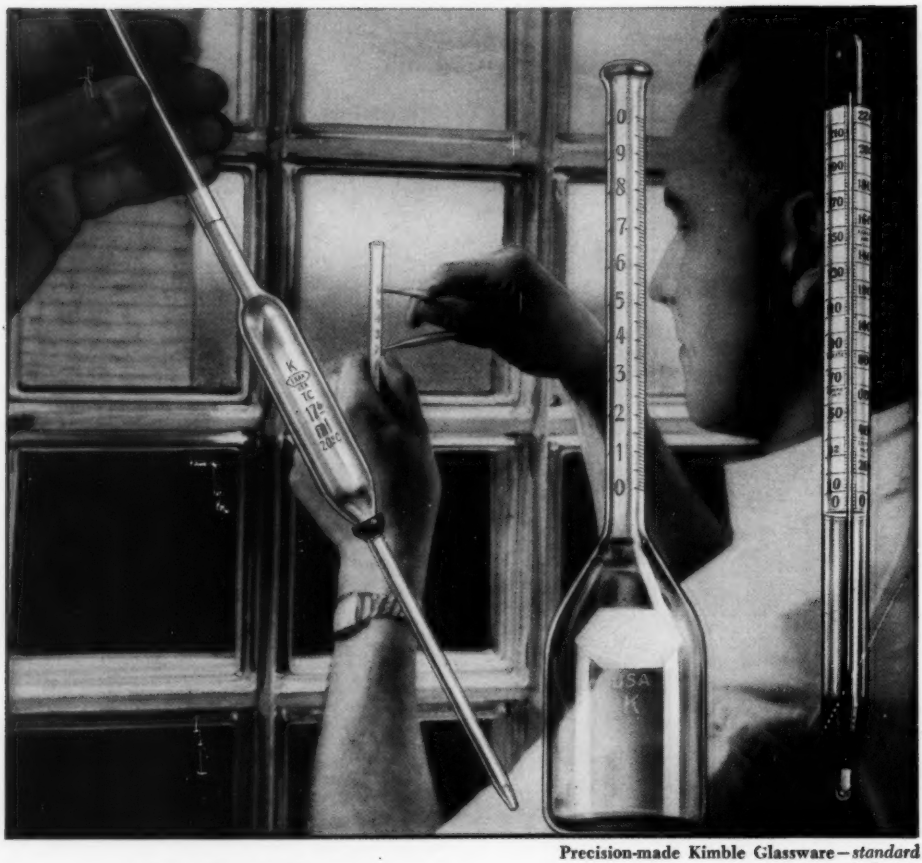

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PEOPLE AND EVENTS

The President Comments

Beginning with this issue of our Journal I'd like to visit with you briefly each month about activities and affairs of our Association.

All committees have been appointed and are functioning in their many areas. When you see the list of these committees in the January issue of the Journal you will appreciate more fully the large number of your fellow members who are giving freely and unselfishly of their time and talents in carrying on the work of our Association.

K. L. Turk

Membership. First of all, I should like to urge each and every one of you to participate in our membership campaign, which got under way Oct. 1. The Membership Committee, under the chairmanship of J. B. FRYE, JR., has done a fine job of getting this campaign under way. A goal of 2,500 members has been established for 1959. That should not be difficult if we all do our part. Dr. Frye has worked out a very complete organization with regional chairmen and also a chairman for each state.

Actually, every one of us should consider ourselves part of the membership committee. We come in contact almost every day with potential members. Personal contacts with prospective members are far more important than letters. Make it a point to telephone or call on prospects in your area. Send the names of prospective members to your state chairman or to a member of the Committee.

Our efficient Secretary-Treasurer, H. F. JUDKINS, has made it easy for everyone to nominate members this year. You will find on your dues notice a place provided for you to nominate one or more prospective members. Take advantage of this opportunity and let's all help to put our membership drive over the top.

Pay your dues promptly. Did you read the pink card you received recently from Mr. Judkins, which said "Please!" at the top? If not, read it right now and see what you can do to save time and trouble for our Secretary, for the Journal printers, and for you. So, pay your dues promptly this year—before Jan. 1, 1959—please!

Association Award nominations. One of the highlights of our annual meeting each year is the recognition of those who receive awards for meritorious achievements in research, teaching, and extension.

Along with your dues notice, you received a yellow blank which provides you with an opportunity to nominate your fellow members for one of these awards. I urge you to give careful consideration to this and nominate candidates who have done really outstanding work and are definitely entitled to the award if chosen. We have many highly deserving people in our Association who have not won any of these major awards.

Please don't hesitate to nominate a candidate just because you are afraid you might have to assemble the dossier material in support of your nominee. Let's see that every really qualified candidate is nominated.

We have a new award this year for the first time. It is the Paul Lewis Laboratories Award for outstanding research dealing with cheese. It will be given to someone who has made a meritorious scientific contribution or discovery relating to chemistry, biochemistry, bacteriology, engineering, or technology pertaining to the cheese industry.

Program Committee. Your Program Committee, under the leadership of F. J. BABEL, is now planning for the biggest and best annual meeting ever at the Univ. of Illinois, Urbana, June 15–17, 1959. The program planning meeting will be held in Chicago the latter part of November. If you have comments or ideas on the general program of the Association annual meeting or symposia topics, please pass them along to your Section Chairman.

As a final thought this month, I should like to invite each one of you to send your comments and recommendations on any items that will help make a better American Dairy Science Association. This is your organization, and free communication and discussion are welcomed by your officers.

K. L. TURK

Nominations for Vice-President and Directors of A.D.S.A.

Each year the members of A.D.S.A. elect a vice-president and two directors. The Nominating Committee selects two candidates for each office to give proper presentation to all fields of interest, according to the provisions of Article II, Section 3, of the By-laws.

Members of the Nominating Committee, realizing the importance of their decisions, have expressed the need for more information on proper qualifications of candidates for these offices. The Policy Committee has studied this problem and has submitted certain suggestions to the Executive Board. These suggestions, modified and approved by the Board, are published here by the Executive Board, so that every member so desiring may know how he can assist in this important selection of candidates.

The Policy Committee believes that mem-

bers who have served on Association committees or as officers and committee workers in sections and divisions, have shown more than ordinary interest in Association work. Because of their services to the Association, they are usually well-known. They have had an opportunity to demonstrate to fellow members their ability and leadership and their willingness to give their time to work on behalf of the Association. These experienced workers should be especially well-qualified to become candidates for nomination to the Association offices of greater responsibility.

Article II, Section 2, of the By-laws indicates how the members of the Nominating Committee should be chosen. In selecting them it would seem wise, if possible, to appoint men who may have held offices in sections and divisions, because of the probability of their broader acquaintance among fellow members. But, having served a term on this committee, members should be excused from appointment, at least for a few years, in order that they may not be excluded automatically from the list of eligible candidates.

In choosing each candidate for vice-president certain desirable qualifications, in addition to personal ability, might be considered. Some of these qualifications include service as Director; experience on the Journal Management Committee or the Editorial Board; work on Association Committees, particularly those concerned with administrative and financial problems; a record of activities as officer and committee worker in section or division; administrative experience outside of the Association and, finally, a willingness to devote to the Association the considerable time and energy required by the responsibilities of the Vice-President's office and, later, the President's office.

Candidates for Directors might well be chosen after having somewhat similar experiences, which should have given them an insight into some of the financial, administrative, and organizational problems of the Association.

Procedure for nominations. (1) Each year the October JOURNAL OF DAIRY SCIENCE shall carry a notice from the Chairman of the Nominating Committee inviting members to suggest names of suitable persons for the offices of Vice-President and Directors. The notice shall indicate from what broad fields of interest the candidates are to be chosen according to the instructions of the By-laws.

(2) These suggestions, as well as those from all members of the Committee, should reach the Chairman by Jan. 1. The Chairman may solicit other suggestions if he desires to do so.

(3) Having received these suggestions, the Chairman will then ask each nominator to secure, confidentially if possible, biographical data concerning his chosen nominee, and to send to the Chairman five duplicate copies of these data on forms provided by the Chairman for that purpose.

(4) The Chairman shall then prepare a list of candidates for each office and send copies of this list, along with the biographical data on the candidates, to each Committee member. The Committee member will place names for each office in the order of his choice and return the list to the Chairman. Successful candidates chosen must receive the approval of the majority of the Committee. Revised lists of candidates shall be resubmitted, if necessary, by the Chairman to the Committee, until approval of the majority has been obtained. The Chairman will then ask the first nominee on the list approved by the Committee for each office to accept the nomination. If he refuses, the Chairman will extend the invitation to the next ranking nominee on the Committee's list for that office, and so on, until a candidate is chosen.

(5) The Chairman of the Committee shall send the names of the men eventually nominated for each office, together with their photographs and biographical sketches, to the Editor-in-Chief for publication in the April issue of the JOURNAL OF DAIRY SCIENCE. The Chairman shall also send a duplicate copy of names of nominees to the Secretary.

(6) Ballots will be mailed to members by the Secretary, the votes tallied, and the results announced according to the provisions of Article II of the By-laws.

W. M. ROBERTS

Burgwald Named Superintendent of Students' Dairy Judging Contest

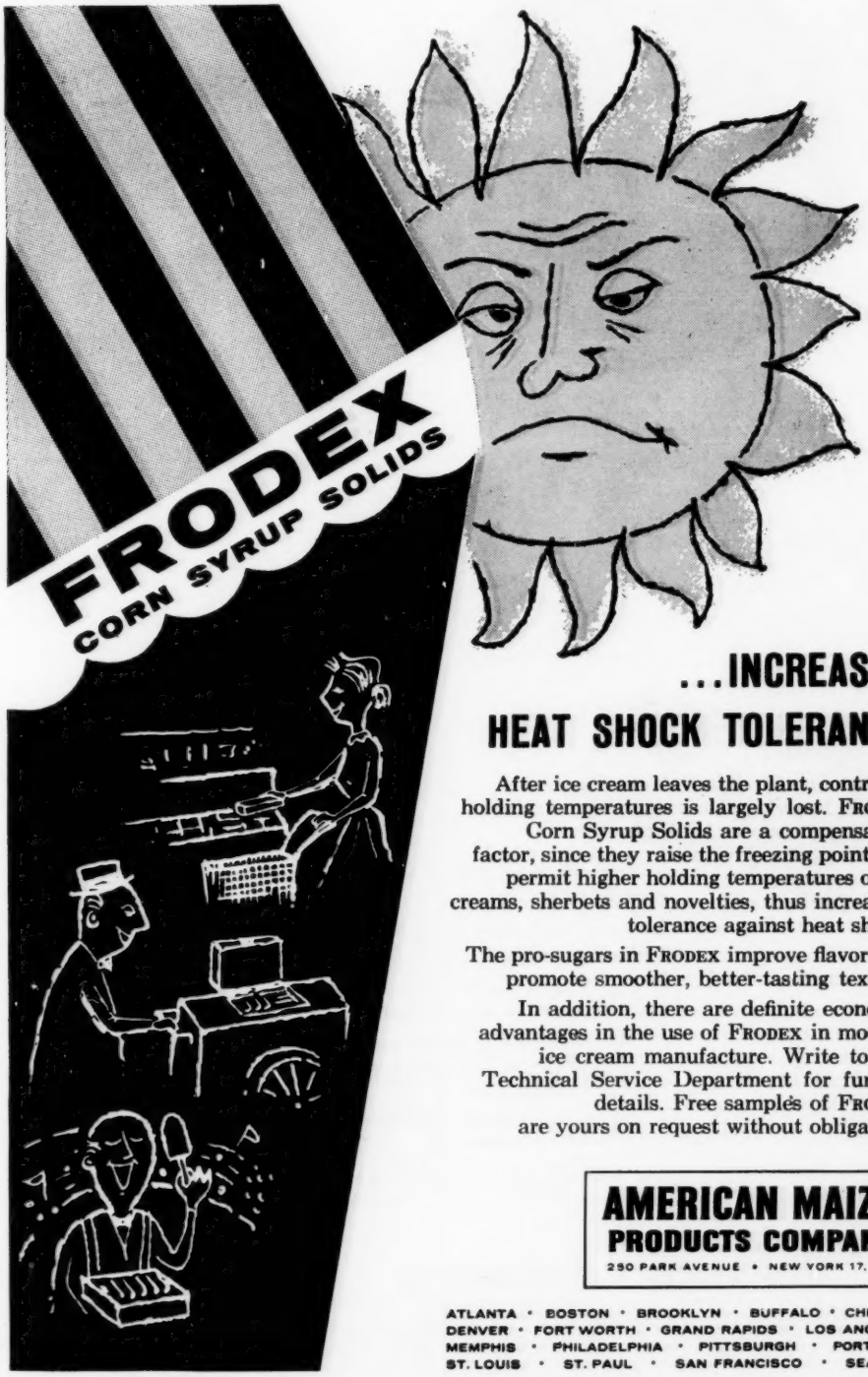
L. H. BURGWALD, marketing specialist with the Dairy and Poultry Division of the Foreign Agricultural Service, USDA, has been named Superintendent of the annual Collegiate Students' Contest in Judging Dairy Products, which will be held Dec. 8, 1958, in the Chicago area.

L. H. Burgwald

D. A. Pettee

Prof. Burgwald succeeds the late C. J. BABCOCK, former chief of the same USDA division, who was Contest Superintendent from 1941 until his death early in 1958.

The contest was started in 1916 by the A.D.S.A., and has been co-sponsored since 1930



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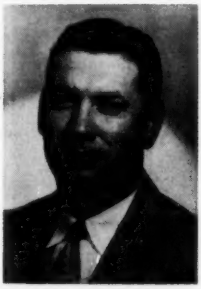
Before accepting his present post with the USDA, Prof. Burgwald was a member of the Ohio State Univ. faculty for 26 yr., and coached several Ohio State dairy products judging teams for Contest participation.

Further experience which Prof. Burgwald brings to his new association with the Contest includes that gained in supervising student milk judging for Ohio State's own contest for vocational agriculture students from 50 high schools. He also has served as assistant chairman and chairman of the dairy manufacturing section of A.D.S.A.

The Contest and the traditional awards banquet, which will be held Dec. 9, will be events of major significance during a full week of dairy industrial activity centering in Chicago. From Dec. 8 through 13, the D.I.S.A.-sponsored 21st Dairy Industries Exposition will transform Navy Pier into a modern wonderland of displays, featuring the latest in supplies, equipment, and services for the dairy industries. During the course of the Exposition, the annual conventions of national and international dairy industry organizations will be held. Organizations include the Dairy Suppliers' Foundation, Dairy Society International, Milk Industry Foundation, National Association of Retail Ice Cream Manufacturers, National Ice Cream Mix Association, International Association of Ice Cream Manufacturers, Evaporated Milk Association, and American Butter Institute.

J. H. Erb



D. R. Strobel

Aims of the Contest are to attract young men and women of high caliber to the dairy industries, to emphasize the importance to the dairy industries of high-quality dairy products, and to foster research into dairy problems—aims which have been strengthened since 1930 through the offering by D.I.S.A. of graduate research study fellowships to the three highest scoring teams in the Contest's All-Products Division.

Top award is $1,800; second place, $1,650, and third, $1,500, with selection of the individual students who receive the awards being left to the faculties of the winning colleges. Fellowship winners then choose from among the colleges represented in the current Contest those at which they wish to do graduate study —this subject to guidance by the A.D.S.A.—D.I.S.A. Committees, which also approve the selections of problems. Scientific information developed by the Fellows subsequently is made available to the entire dairy field.

To date, 89 fellowships have been granted, resulting in valuable contributions to dairy science and technology, and giving added spur to the development of dairy leadership. Many former Contest fellowship winners are today key dairy figures in fields of industry, government, and education.

Other tangible rewards for Contest winners include a silver bowl for the top team in the All-Products Division, and silver cups for highest scoring teams in Milk, Butter, Cheese, and Ice Cream Divisions. Donors are: All-Products Bowl, and Butter and Cheese Cups, D.I.S.A.;

H. L. Wilson

N. E. Fabricius

Milk Cup, Milk Industry Foundation; Ice Cream Cup, International Association of Ice Cream Manufacturers. These are perpetual trophies which must be won three times by one college before they may be kept permanently.

In addition, fine watches are presented to first-place individual winners in the five Contest divisions; to the second- and third-place individuals go, respectively, sterling silver and bronze medals imbedded in lucite from the following donors: All-Products, D.I.S.A.; Butter, American Butter Institute; Milk, M.I.F.; Ice Cream, I.A.I.C.M.; and Cheese, National Cheese Institute.

Land grant colleges and universities and corresponding institutions in the United States and other countries are eligible to enter three-man teams of junior or senior dairy manufacturing students in the Contest. (The all-time record number of participating teams, 33, was established in 1956.) Team members are picked for their general scholastic ability and aptitude for research, as well as for their proficiency in judging dairy products. They must never have taken part in any other national or international dairy products judging contest, have acted as official judges of dairy products, or taught the manufacturing or judging of dairy products.

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During the Contest, students are divided into four groups, alternating at the milk, butter, cheese, and ice cream tables in innings of 40 min. each, with 5-min. breaks between innings. Ten samples of each product, ranging from poor to excellent quality, are taste-tested by each contestant. Eighty-one decisions on fine points of quality must be made and entered on special score-cards for each sample of milk and ice cream; 80 for butter and cheese.

Before the Contest, product samples are scored by the official judges, whose evaluations then are double-checked by two coach judges, who are appointed by the A.D.S.A.—D.I.S.A. Contest Committee or elected by the participating coaches, as well as by a D.I.S.A. Associate Judge.

Serving with Prof. Burgwald as officials of the 1958 Contest are D. R. STROBEL, Chief, Foreign Marketing Branch, Dairy and Poultry Division, Foreign Agricultural Service, USDA, assistant superintendent; D. A. PETTEE, The Creamery Package Mfg. Company, milk judge; H. L. WILSON, Consolidated Dairy Products Company, cheese judge; J. H. ERB, The Borden Company, ice cream judge; N. E. FABRICIUS, Flambeau Valley Farms Cooperative, butter judge.

D.I.S.A. Associate Judges are DAN ROAHEN, The Creamery Package Mfg. Company, milk; NEIL ANGEVINE, Meyer-Blanke Company, cheese; VERN WILT, The Liquid Carbonic Corporation, ice cream; CHARLES WEINREICH, Cherry-Burrell Corporation, butter. Mr. Weinreich also serves as the D.I.S.A. Chairman of the Contest and Fellowship Committee. G. M. TROUT, Michigan State Univ., is the A.D.S.A. Chairman.

Zittle To Receive '59 Borden Award

The Borden Award in the chemistry of milk, consisting of $1,000 and a gold medal, will go next year to C. A. ZITTLE, research biochemist of the Eastern Utilization Research and Development Division, USDA, Philadelphia. The award is in recognition of his basic research in the proteins of milk.

C. A. Zittle

A native of Lancaster County, Pa., Dr. Zittle received his B.S. degree from Franklin and Marshall College and his Ph.D. degree from the Univ. of California. After a few years of teaching, he joined the research staff of the Univ. of Pennsylvania Medical School, where he conducted research on hemolytic streptococci and bovine spermatozoa. He subsequently served as head of the immunochemistry division in the research laboratories of a large pharma-

ceutical company and coordinator of enzyme research at the Biochemical Research Foundation, Newark, Del. He joined the USDA in 1948. Dr. Zittle has authored more than 60 scientific articles in his various research fields.

The Borden Award in the chemistry of milk is administered by the American Chemical Society, which selects the outstanding research scientist in the field. The winner is announced at the Society's fall meeting and the award is presented at the following spring meeting. The award has been made annually since 1939.

New Food Additives Law Enacted

A new and important amendment to the Federal pure food and drug law went into effect in September when President Eisenhower signed a bill requiring the safety of food additives to be established before such chemicals can be used in food or feed for humans or animals.

Under this amendment to the Federal Food, Drug, and Cosmetic Act, the manufacturer or promoter of a new food additive will have to test it on laboratory animals and submit the test results to the FDA. If the FDA is satisfied that the data establish the safety of the additive under proper conditions of use, it will issue a regulation specifying the amount which may be used, the foods in which it may be used, and any other necessary conditions of use.

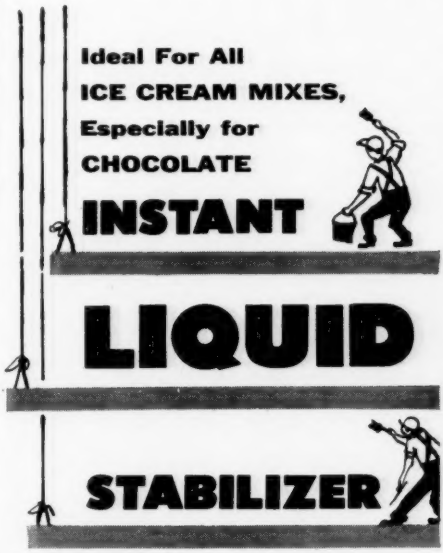
If, in the opinion of the FDA, the safety of the additive is not established by the data, its use will not be permitted.

A Message from Your National Membership Committee

Your National Membership Committee wishes to express its sincere appreciation to all members of the Membership Committee at the division and state levels, to department heads, to various officers of the A.D.S.A., and others who have been instrumental in the success of the membership drive during its initial phases. Although we have gotten off to a good start, a great deal of work remains to be done between now and the termination of the student affiliate drive on Dec. 15, 1958, and the regular membership drive on Jan. 1, 1959.

It is extremely important for the Association that present members renew their membership on or before Jan. 1, 1959. If this is not done, your Secretary, H. F. Judkins, must send a written notice to the printers of the Journal who, in turn, will remove the names of unpaid members from the mailing list. When the membership is finally paid, the entire process must be repeated in the reverse manner.

You would do your Association a real service if you would be so kind and thoughtful as to renew your membership on or before the deadline. Mr. Judkins has emphasized rather strongly that he would be extremely happy if the membership would cooperate on this matter.



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Chicago Dairy Tech Society Awards Three Scholarships

The highlight of the Chicago Dairy Technology Society's September meeting, held at their Furniture Mart clubroom in Chicago, was the presentation of three $1,000 Scholarship Awards.

The award winners this year were JAMES LESCOHIER of Fort Atkinson, Wis.; CALVIN DEWSNAP of Endeavor, Wis., and RAYMOND WOODEN of Bradford, Ill. Both Dewsnap and Lescohier plan to enroll at the Univ. of Wisconsin; Raymond Wooden is attending the Univ. of Missouri.

Top row, left to right, Charles Miller, president; J. H. Petersen, Treasurer; D. R. Olson, chairman, Education Committee, and Joseph Tobias, secretary. Bottom row, James Lescohier, Ft. Atkinson, Wis.; Calvin Dewsnap, Endeavor, Wis.; and Raymond Wooden, Bradford, Ill.

D. R. OLSON, Chairman of the Society's Education Committee and Sales Manager of the Krim-Ko Corporation, pointed out the importance of this award to the boys, and acknowledged that he received the first scholarship award given by the Chicago Dairy Technology Society. The Society awards three scholarships annually. Students selected will receive $250.00 annually for 4 yr. Prerequisites for the scholarship are that the student's parents must live in Illinois, Iowa, Indiana, Michigan, or Wisconsin; that students must enter a college that carries an accredited curriculum in the dairy industry, dairy manufacturing, or dairy technology. All applicants are screened on the basis of scholastic standing, extracurricular activities, and general character.

The principal speaker of the evening was A. H. WAKEMAN, Director of Research for The Creamery Package Mfg. Company, who discussed "Cleaned-In-Place Procedures."

Ohio State News

The Tenth Annual Dairy Technology Homecoming Brunch was held Oct. 25 in the new Agricultural Administration Building at Ohio State Univ., Columbus.

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The general theme at the October meetings of the Ohio Dairy Technology Societies was "The Future of Urban Growth and the Dairy Industry." Speakers at the various societies were R. L. FREDERICKS, president of the Timken Roller Co., Canton, O., who spoke at Cleveland; HERBERT STEVENS, director of the City Planning Commission, Cincinnati, who spoke at Cincinnati; and T. K. HAMILTON, president of the Diamond Milk Co., Columbus, who spoke at Columbus.

Winners in the various classes in the Dairy Products Division of the 1958 Ohio State Fair were Pearl Valley Dairy, Pearl, for Swiss cheese in wheels; Masts Dairy, Walnut Creek, for Swiss cheese in block; Barnesville Dairymen's Cooperative Association, Barnesville, for cottage cheese; BUD BALLARD, West Jefferson, for milk judging without formal training; J. O. ROBERTS, Columbus, for milk judging with formal training, and ROBERT MILKIE, Clyde, for ice cream judging.

Official judges were R. E. HARDELL, Monroe, Wis., for Swiss cheese and J. M. JENSEN, Michigan State Univ., East Lansing, for ice cream and cottage cheese.

The entire operation of the Dairy Industry Exhibit was under the supervision of the Dept. of Dairy Technology, such as the development of educational exhibits and arrangement, and supervision of dairy products and judging contests, and sales of dairy products. Special exhibits were arranged by the A & P Food Stores, Columbus, and the Ohio Division of the American Dairy Association.

A series of five Fieldmen's meetings were held Oct. 20-24 at Dayton, Findlay, Columbus, Cambridge, and Akron.

A one-day program was held in each city, which included discussions on feeding, production, milk quality, communication, and economics. In addition to local county agents and fieldmen, the following Ohio State Univ. specialists appeared: LESTER ARNOLD, NEAL CARPENTER, FRANK KOVAL, E. ROGERS, and ELMER BAUMER.

The meetings are sponsored jointly by the Dept. of Dairy Technology, Ohio State Univ., and the Ohio Dairy Products Association.

Illinois News

P. H. TRACY, professor of dairy technology at the Univ. of Illinois, will spend a six-month sabbatical leave studying the impact of automation in the dairy industry. He began his study Sept. 1, 1958, and will conclude it in March, 1959.

Dr. Tracy will study the effect of automation upon the amount of milk produced per farm; number of producers; quality of raw milk received; plant layout and design; milk handled in plants per man-hour; total labor; quality and uniformity of products produced; extent

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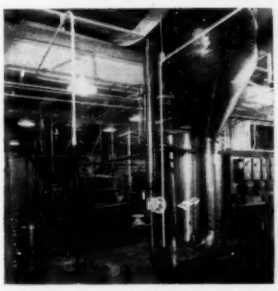

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of adaptation as related to location, size, and type of plant and the products produced; costs; type of workers employed; organized labor attitudes, and future location of plants.

Dr. Tracy will spend most of his time visiting plants in Chicago, Cleveland, Detroit, Denver, Salt Lake City, Seattle, Portland, San Francisco, Los Angeles, Phoenix, Tucson, San Antonio, New Orleans, Miami, St. Petersburg, Atlanta, Philadelphia, New York, Boston. and Minneapolis.

According to R. T. MILNER, head of the Dept. of Food Technology, Dr. Tracy's study on automation will be partially supported by the Agricultural Marketing Service of the USDA. D. R. MOORE, who earned his M.S. degree at the Univ. of Illinois in 1951, will assist Dr. Tracy in his work.

T. T. THOMPSON, sales manager for the Dean Milk Company, Louisville, Ky., has been assigned to supervise sales in Flint, Mich., in addition to his sales work in Kentucky.

Mr. Thompson started with the Dean Milk Company in 1948 after graduating from the Univ. of Illinois in dairy technology. He was a salesman in the ice cream and fluid milk divisions before taking his present position in Louisville in 1952.

LAUREN DUNAWAY has been promoted to the position of quality control supervisor for the Dean Milk Company in Flint, Mich. Mr. Dunaway graduated from the Univ. of Illinois in June, 1958, in dairy technology. Before graduating, he worked summers in the company's plants at Rockford and Pecatonica, Ill.

J. R. LATHROP, JR., has been transferred to Louisville, Ky., where he will be in charge of milk sales for the Dean Milk Company. A graduate of the Univ. of Illinois in dairy technology, Mr. Lathrop joined Dean's staff in 1955 and worked as a milk solicitor in Chicago.

Pennsylvania News

R. D. MCCARTHY has joined the staff of Pennsylvania State Univ., to do research under a grant from the National Heart Institute of the National Institutes of Health. He will be a member of the Dept. of Dairy Science.

Dr. McCarthy earned his B.S. and M.S. degrees in dairy science at Penn State and his Ph.D. at the Univ. of Maryland, College Park. His work at Pennsylvania will deal mainly with research on the analysis and biogenesis of milk fat.

Maine News

M. J. ANDERSON has been named Assistant Prof. of Animal Science at the Univ. of Maine, Orono. He is a native of Heber, Utah, and has completed his doctoral work in animal nutrition at Cornell Univ., Ithaca, N. Y.

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Florida News

L. R. ARRINGTON, associate professor and associate animal nutritionist at the Univ. of Florida, Gainesville, will spent October, November, and part of December in Ethiopia participating in a nutrition survey in that country.

The survey is sponsored by the Interdepartmental Committee on Nutrition for National Defense and will be similar to surveys which have been conducted in other countries. Survey work will be conducted by a team of nutritionists, a food technologist, physicians, dentists, and a biochemist.

Louisiana News

A one-day short course for milk-tank truck operators was held at Louisiana State Univ., Baton Rouge, in August. Thirty-five people registered for the course, which was under the direction of A. J. GELPI.

The program dealt with such topics as the detection of off-flavors, proper procedures for sampling for fat content and bacteria count, and milk sanitation in general.

L. L. RUSOFF, who was chosen by the chairman of the Board of Supervisors, to serve on a University-wide Centennial Committee, has assumed the duties of chairman of the science exhibits.

N.A.A.B. Convention Report

More than 600 people from all parts of the U.S., and from Canada, Puerto Rico, and Iran, attended the 11th Annual Convention of the National Association of Artificial Breeders. The meeting was held during August in New Orleans, La.

New officers elected are RICHARD KELLOGG, manager of the Central Ohio Breeding Association, president; CHARLES KRUMM, manager of the Southern Wisconsin Breeders Association, Madison, vice president; H. A. HERMAN, executive-secretary of the N.A.A.B., Columbia, Mo., secretary-treasurer.

J. N. EFFERSON, dean of the College of Agriculture, Louisiana State Univ., gave an address on "The Place of Agriculture in an Expanding Industrial Economy." Former President J. S. EARL, who has now retired, and Executive-Secretary Herman emphasized a gain of some 6% in total cows inseminated during 1957. The total was more than 6,000,000 cows, including about 600,000 services to beef bulls.

Other speakers included JUSTIN WILSON, Baton Rouge; MANUEL GORDEN, Dept. of Dairy Husbandry, Michigan State Univ., East Lansing; ALEXANDER SAS JAWORSKY, Ukrainian refugee and now a veterinarian in Abbeville, La. (Mr. Jaworsky achieved national fame by winning $64,000 on the "$64,000 Question" television program); B. J. BURCH, manager of Sontheimer Farms, Hammond, La.; R. E. HODGSON, chief of the Animal Research Branch of the USDA; J. F. KENDRICK, D.H.I.A. and Sire Proving Division of the USDA; K. B. MUSSER, secretary of the Purebred Dairy Cattle Association, Peterboro, N.H.; CHARLES KRUMM, Southern Wisconsin Breeders Cooperative, Madison, and KENNETH MCFARLAND, General Motors Corp.

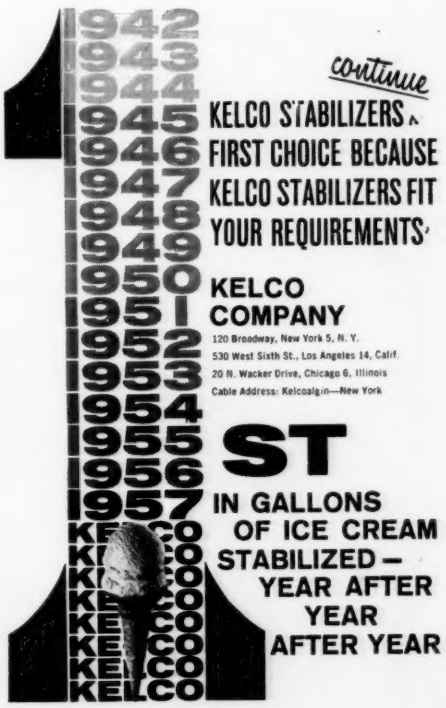
Hosts at the convention were the Louisiana Artificial Breeding Cooperative, Baton Rouge, the State Dept. of Agriculture, and other dairy industry organizations in Louisiana.

Fisher, Goslee Honored at Sanitarians' Convention

Two men received signal honors for long service to International Association of Milk and Food Sanitarians at the group's 45th Annual Meeting held in September in New York City.

M. R. FISHER, D.V.M., Chief of the Milk Control Section of the St. Louis, Mo., Health Division, was presented with the Citation Award, bestowed annually on a member of the association whose contributions over a period of years have furthered the professional advancement of IAMFS and have strengthened its growth and enhanced its reputation.

Dr. Fisher has been a past president of both International Association of Milk and Food Sanitarians and its affiliate, Missouri Associa-



tion of Milk and Food Sanitarians. He was a major architect of the present organizational structure of the International as a member of its Executive Board, and he currently serves on its Committee on Sanitary Procedures, which presents points of view of sanitarians in formulating 3-A Sanitary Standards for Dairy Equipment.

H. C. GOSLEE, Chief of the Dairy Division and Clerk of the Milk Regulation Board, State of Connecticut, Hartford, was made an Honorary Lifetime Member of IAMFS for his long and faithful service both to the International and to its affiliate, Connecticut Association of Dairy and Food Sanitarians, of which he has served as secretary since 1929.

Quaker Appoints Morrison

R. A. MORRISON has been named manager of the Lawrenceburg, Ind., feed sales division of the Quaker Oats Company, succeeding R. B. BENNETT, who has resigned.

At the time of his appointment, Mr. Morrison was serving as manager of feed sales training for the company.

A native of Greenacres, Wash., he received his undergraduate and graduate training at Washington State College. Before joining Quaker, he had been an extension dairyman at Ohio State Univ. He is a member of the A.D.S.A.

Owens-Illinois Promotes Wilhelm, Davis

R. B. WILHELM, for many years one of glass' leading representatives in the dairy packaging field, has been promoted to manager of processed food sales for Owens-Illinois Glass Company.

R. B. Wilhelm

R. E. Davis

Mr. Wilhelm leaves the position of manager of O-I dairy container sales to direct sales in the segment of the business which includes such products as baby foods, fruits, vegetables, tomato products, and juices. He will retain responsibility for sales of containers for instant products, such as coffee and malted milks.

R. E. DAVIS, field manager for dairy contain-

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er sales since earlier this year, has been promoted to the position held by Mr. Wilhelm.

Mr. Davis, a graduate in dairy science from Pennsylvania State Univ., has been associated with dairy container sales since joining Owens-Illinois in 1954.

Mr. Wilhelm is a member of the Quarter Century Club of O-I and has been manager for many years of the dairy container sales division and more recently also of the instant products division. In that span he has assisted in making many valuable contributions by Owens-Illinois to the glass packaging of fluid milk. During the years, he has seen the weight of the quart milk bottle reduced from 25½ to 17¾ oz., and the diameter of the top opening narrowed from 56 to 38 mm.

Other changes have included the introduction of handi-square bottles, Applied Color Lettering labels, and the trend toward gallon and half-gallon containers.

Active in many dairy industry organizations, Mr. Wilhelm is director and vice-president of the Dairy Industries Supply Association, and is a past director of the National Dairy Council.

Diehl Retires from Refrigeration Foundation

H. C. DIEHL, director of the Refrigeration Research Foundation, has retired from the position he has held since 1943. W. A. MACLINN, chairman of the Dept. of Food Science at Rutgers Univ., New Brunswick, N. J., has been named to take his place.

Dr. Maclinn received his Ph.D. from the Univ. of Massachusetts in 1938 and during World War II was executive officer and assistant director of the Food and Container Research Institute for the Armed Forces in Chicago. He joined the Rutgers staff in 1946, and initiated a program of research and teaching in the field of food science and technology.

Borden's Increases Research Staff

The Borden Company announces the addition of seven scientists to its technical research staff at the company's Research and Development Center at Syracuse, N.Y.

They are D. L. ANDERSON, Cedar Rapids, Ia., M.S. from the Univ. of Wisconsin; K. W. HENRY, State College, Pa., B.S. from Pennsylvania State Univ.; P. C. HINTZ, Greenville, Pa., M.S. from Ohio State Univ.; C. L. HANKINSON, Columbus, O., Ph.D. from the Univ. of Minnesota; D. R. MORGAN, Madison, Wis., Ph.D. from the Univ. of Wisconsin; P. M. MONTELEONE, B.S. from LeMoyne College, Syracuse; and A. K. MOZINGO, Unadillo, N.Y., M.S. from the Univ. of Wisconsin.

Kansas City News

The Kansas City Area Dairy Technology Society held its annual Dinner-Dance in October. This dinner-dance is an informal event to kick off the Society's 1958-1959 season.

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Western Condensing Co. Promotions

Two recent promotions have been announced by the Western Condensing Company. They are J. V. REGER, who was named sales manager for all five grades of Foremost Lactose on a national basis, and E. A. AELSCH, who was named sales manager for Valley Fresh Sweet Dairy Whey and allied milk ingredients.

Mr. Reger joined Western's food research department upon graduation from the Univ. of Minnesota, where he received his B.S. and M.S. degrees in dairy products manufacture. He was later transferred to technical sales and service and then promoted to assistant sales manager of the foods products division in 1955.

J. V. Reger

E. A. Aelsch

Mr. Reger is an active member of the A.D.S.A., the Institute of Food Technologists, the American Association of Cereal Chemists, and the American Association of Candy Technologists.

A graduate of Dunwoody Institute and the American Institute of Baking, Mr. Aelsch has been associated with the baking industry for the past 24 yr. After his discharge from the U.S. Navy in 1947, Mr. Aelsch joined Western's staff to work on new product developments. He then serviced accounts nationally as assistant sales manager of the food products division.

He has been a pioneer in the development of whey ingredients the past 10 yr.

Beatrice Promotes D. L. Chew

D. L. CHEW has been named manager of the St. Joseph, Mo., plant and branches of Beatrice Foods Co., Chicago. Mr. Chew, a graduate of Kansas State College, Manhattan, has been assistant manager of the St. Joseph plant since 1957.

He had been owner and manager of the Harris Dairy Co., St. Joseph, until it was merged with Beatrice in 1954. He attended the Atchison, Kan., public schools and, during World War II, served with the Eighth Air Force in the South Pacific.

F. W. Barber Heads Sanitarians

F. W. BARBER, National Dairy Research Laboratories, Oakdale, L.I., N.Y., has been elected president of the International Association of Milk and Food Sanitarians. He was elected at the 45th Annual Convention of the group, held in New York in September.

Other officers elected are W. V. HICKEY, New York, president-elect; J. J. SHEURING, Univ. of Georgia, first vice-president; CHARLES WALTON, Laramie, Wyo., Health Dept., second vice-president; V. T. FOLEY, Kansas City, Mo., secretary-treasurer. H. L. THOMASSON, Shelbyville, Ind., is executive secretary.

More than 600 sanitarians attended the three-day session which was held jointly with the regular meeting of the New York State Association of Milk Sanitarians and the Cornell Dairy Industry Conference. The 1959 meeting will be held Aug. 26-28 in Glenwood Springs, Colo.

Completed Theses

Ph.D. Degree

R. D. MCCARTHY—Tracer studies of the perfused rumens and livers of goats. Univ. of Maryland, College Park.

M.S. Degree

W. H. FEASTER—The effect of high and low protein concentrate on milk production and various blood constituents. Univ. of New Hampshire, Durham.

J. B. HOLTER—Determination of acetic, butyric, and valeric acids in bovine blood by isotope dilution. Univ. of Maryland, College Park.

Smith Promoted by Cabell's Milk Co.

L. B. SMITH has been named director of operations for Cabell's Dallas milk plant after seven years as superintendent of the plant. He succeeds the late JULIAN WILLIAMS.

DONALD PRINE, who joined Cabell's 3 yr. ago as supervisor in the milk processing department, is the new milk plant superintendent.

Mr. Smith is a graduate of Mississippi State College. Before joining Cabell's in 1951 he was superintendent of the dairy plant and instructor in dairy manufacturing at Texas Tech. He is vice-president of the Dallas–Fort Worth Dairy Technology Society and a member of the standards committee of the Dairy Products Institute of Texas.

News from Connecticut

R. G. JENSEN, Assistant Professor of Dairy Manufacturing, has received a research grant of $21,240 from the Public Health Service to study the "Hydrolysis of Selected Triglycerides by Milk Lipases." M. E. MORGAN, Professor of Dairy Manufacturing, will cooperate in the project, which will continue for 3 yr.

Pennsalt Names Four Sales Representatives

Pennsalt Chemicals Corporation announces the appointment of four new sales representatives. They are J. R. COX, E. T. BATISTA, D. R. TREPTOW, and MERLE VANDENBURG.

Mr. Cox will cover the Kentucky territory, with headquarters in Louisville. He is a graduate of Lincoln Chiropractic College and also attended the Univ. of Louisville.

Mr. Batista will take over the western New York State and western Pennsylvania territory. He attended Heidelberg College, Tiffin, O., and Capital Univ., Columbus, O. His headquarters will be in Springfield, N.Y.

Mr. Treptow, a graduate of the Univ. of Wisconsin in dairy industry, will cover the Iowa territory, with headquarters in Des Moines.

Mr. Vandenburg is a graduate of Hope College, Holland, Mich., and will cover the Ohio territory from headquarters in Findlay, O.

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THE MINOR PROTEINS OF BOVINE MILK

R. McL. WHITNEY
Department of Food Technology, University of Illinois, Urbana

SUMMARY

To summarize the state of knowledge concerning the minor proteins of bovine milk, it can be observed that:

1. The following proteins have been adequately shown to be native constituents of milk, prepared in crystalline, or very pure form, and some knowledge is available concerning their composition, structure, and properties: xanthine oxidase, lactoperoxidase, alkaline phosphatase, and trypsin inhibitor.
2. The following proteins have been adequately shown to be native constituents of milk and some knowledge is available concerning their properties: protease, amylases, lipases, and lactenins I and II.
3. The following proteins have been shown to be present in milk and have been prepared in a crystalline or concentrated form, although they still appear to be heterogenous and some question exists as to their origin: γ-casein, and a red protein.
4. The following proteins have been shown to be present in milk, but a question exists as to their origin, and they have not been adequately purified for characterizations: the lactose-destroying enzymes, aldolase, catalase, copper protein, σ-proteose, and sunlight-flavor preeursor.

That much more information is needed is obvious.

For purposes of this review, the minor proteins of milk are considered to be all of the proteins present at such concentrations as to be undetected electrophoretically, either in the skimmilk or in the whey, at a protein concentration of 1% at pH 8.7 in veronal buffer of ionic strength of 0.1. In other words, they may be considered as all other proteins that are not listed by the report of the American Dairy Science Association's Committee on the Nomenclature, Classification, and Methodology of Milk Proteins *(71)*.

These minor proteins are recognized to be a rather heterogeneous group, which have been indicated with varying degrees of certainty to be present in milk. Undoubtedly, they are minor components of the major proteins and are fractionated with them. Wherever possible, the emphasis in this review is laid on the properties of the isolated proteins, and those properties reported are chiefly those indicative of the physical and chemical structure of the protein, such as: chemical analyses, absorption spectra, molecular weights, axial ratios, electrophoretic properties, denaturation conditions, and complex formation. A number of the proteins listed are enzymes but, in reviewing our knowledge of them, their enzymatic activity is discussed only as it supplies us with information upon their physical and chemical structure.

It is the purpose of this review not to tabulate all of the literature references available upon these proteins but, rather, to supply a picture of the current knowledge covering them, with the hope that it will stimulate further research in this area. To emphasize the information which is still needed, the proteins are arranged, to the extent possible, in the order of decreasing quantity of available information concerning them.

XANTHINE OXIDASE

In 1902, Schardinger *(126)* discovered that the reduction of methylene blue by aldehydes was catalyzed by fresh milk. Later, Morgan, Stewart, and Hopkins *(96)* proved that milk was also a rich source of xanthine oxidase. From a study of the various factors affecting these two reactions, some of the workers in the field *(17, 19, 20, 35, 38, 85, 92)* have assumed that the same enzyme is involved in both. However, to prove conclusively this identity, it was necessary to isolate the enzyme. Several investigators *(16, 30, 31, 36, 37, 68, 86, 94, 125)*, in the years following, have succeeded in preparing fractions which were rich in xanthine oxidase and in securing considerable information concerning its chemical composition and properties. Finally, Avis, Bergel, and Bray *(11)* succeeded in crystallizing the enzyme in a high degree of purity, which they have conservatively estimated to be 90% pure.

While earlier investigators had proposed rather convincing evidence that the xanthine oxidase and Schardinger's enzyme were one and the same, Avis *et al. (12)* were able to prove conclusively with their crystalline protein that not only were they the same but that the enzyme could also oxidize reduced diphosphopyridine nucleotide, DPN, with the following systems acting as electron acceptors: oxygen, methylene blue, 2,6–dichlorophenolindophenol, and cytochrome-C. Prior to this, Hofstee *(68)* had advanced strong evidence of the identity of xanthine oxidase and xanthopterin oxidase.

Ball *(16)* and Corran and Green *(31)* demonstrated that one of the prosthetic groups in the molecule was a flavin which was not riboflavin but, on the basis of its phosphorus content, absorption spectra, and coenzyme activity for amino acid oxidase, might be a flavin adenine dinucleotide similar but not identical to the coenzyme of amino acid oxidase.

Based upon the indicated association between molybdenum and xanthine oxidase by DeRenzo *et al. (33)* and Richert and Westerfield *(120)*, Totter *et al. (140)* established that molybdenum was an integral part of the xanthine oxidase, by establishing the constancy of the ratio of Mo^{99} to xanthine-oxidase activity during rigorous purification procedures when the Mo^{99} previously had been injected into the cow intravenously. This was later confirmed by Mackler, Mahler, and Green *(86)* and Avis, Bergel, and Bray *(12)*, who found the ratio of Mo: flavin adenine dinucleotide to be 0.69. However, most investigators found the molybdenum to be loosely bound and Mahler and Green *(87)* suggest that this ratio should be 1:1.

Richert and Westerfield *(121)*, by procedures analogous to those used for molybdenum, showed that iron also was an integral part of the enzyme molecule, and Avis *et al. (12)* found for their crystalline enzyme average ratios for protein: flavin adenine dinucleotide: Mo: Fe of 1:2:(1.3-1.5):8.

Investigations of the absorption spectra by various investigators *(16, 30, 86, 121)* suggest the presence of another chromophoric group, and Richert and Westerfield suggest that it may be due to the linkage of the iron to the protein. However, Morrell *(94)* claims that the increased absorption observed is due to

the manner of linkage of the flavin adenine dinucleotide to the protein. The absorption spectrum *(16)* of the enzyme shows a broad band at 450 mμ, which contributes the red-brown color to the enzyme, a shoulder at 350 mμ, and a sharp band at 275 mμ.

The molecular weight of xanthine oxidase has been calculated in various ways by various investigators, but that calculated by Avis *et al. (13)* from sedimentation, diffusion, and density data on their crystalline material appears to be the most valid. From the extrapolated value of the $S_{20w} = 11.4 \pm 0.3 \times 10^{-13}$ sec.$^{-1}$, a diffusion constant, $D = 3.6 \pm 0.2 \times 10^{-7}$ cm.^{2} sec.$^{-1}$, and a partial specific volume of 0.74 ± 0.02 cc/g, they obtain 290,000 ± 40,000. This agrees with the value obtained by assuming two flavin adenine dinucleotide molecules per unit of protein, 308,000. Assuming no hydration, the axial ratio for the molecule is 7 : 1.

From electrophoretic measurement at 0° C. and 0.2 ionic strength and 0.8% protein concentration, Avis *et al. (13)* established the isoelectric point of xanthine oxidase to be pH 5.3–5.4. While electrophoresis and solubility measurements by the "constant protein" method indicated homogeneity in their purest preparation, sedimentation and solubility measurements by "constant solvent" method indicated the presence of a trace of impurities. However, from a consideration of enzyme activities they concluded that these preparations still consisted of a mixture of two or more very similar entities: *(1a)* the fully active enzyme; *(1b)* the "inactive" enzyme with a modified protein moiety; *(2a)* a molybdenum-poor molecule derived from the active enzyme, and *(2b)* a molybdenum-poor molecule derived from the "inactive" enzyme. It is, of course, possible that all except the fully active enzyme are products of the isolation procedure.

The information available upon the denaturation and other chemical properties of xanthine oxidase is conflicting and needs further clarification with preparations as pure as those of Avis *et al. (13)*. Care needs to be taken also to differentiate between denaturation and dissociation. More information concerning the xanthine oxidase content of dairy products is needed. Greenbank *(52)* estimated on the basis of catalysis of the reduction of methylene blue by hypoxanthine that the xanthine oxidase content of milk averaged 160 mg/liter. More information is also needed upon the distribution of the xanthine oxidase in the milk. While the more recent workers have used buttermilk as the source of their preparations, Dixon and Thurlow *(37)* employed skimmilk with some success. Morton *(104)* found that the lipoprotein particles which he isolated from dialyzed buttermilk contained xanthine oxidase, and Polonovski, Baudu, and Neuzil *(116)* have shown that it is closely associated with the fat-globule membrane.

LACTOPEROXIDASE

The presence of a peroxidase in milk has been known since it was first demonstrated by Arnold in 1881 *(4)*, but it was believed to have originated from disintegration of the leucocytes. Upon the preparation of a highly purified milk

peroxidase by Theorell and Åkeson *(137)*, it was possible to show that this was not the case, since the milk-peroxidase was distinctly different from Agner's *(1)* verdoperoxidase isolated from leucocytes. While Theorell and Paul *(138)* have prepared the lactoperoxidase in crystalline form, they have not as yet published the details of their method and were successful in their preparations only with spring and summer milk. Recently, Polis and Shmukler *(115)* have published the details of a method for the preparation of the enzyme in crystalline form which is successful with milk of all seasons. Morrison and Hamilton *(100)* have also developed a relatively simple procedure for the separation of lactoperoxidase in a fairly pure form with the use of ion-exchange resin.

The absorption spectra of the crystallized lactoperoxidase indicate that it is a heme protein with an iron content of 0.069%, as observed by Polis and Shmukler *(115)*, or 0.0755% according to Theorell and Åkeson *(137)*. The difference in their results may be due to the relative completeness of removal of phosphate ions present in the crystals before analysis. Phosphate ion is known to complex with the lactoperoxidase *(138)*. While qualitatively the absorption spectra obtained by the two investigators are in agreement with weak absorption maxima being observed at ~640, ~600, ~550, ~500 mμ, and strong peaks at 413 and 280 mμ, quantitatively the absorbancy ratios indicated in Table 1 either indicate a difference in purity or a more subtle difference suggested by Polis and Shmukler *(115)*. They were able electrophoretically to separate two enzymatically active peroxidase components from a winter milk, the faster component of which had an absorbancy ratio of $A_{412}:A_{280} = 0.9$; whereas, for the slower component, this value was 0.8. They have designed these components as Lactoperoxidase A and B, respectively. Lactoperoxidase A, upon dialysis to low salt concentration, gradually changes to a protein with spectral constants similar to Lactoperoxidase B.

TABLE 1

Adsorbancy ratios of lactoperoxidase preparations at indicated wave lengths

Enzyme preparation	280 : 250 mμ	412 : 280 mμ	412 : 300 mμ
Polis and Shmukler's *(115)*	2.06	0.90	7.3
Theorell and Pederson's *(139)*	1.85	0.77	5.0

Theorell and Pederson *(139)*, from sedimentation and diffusion data, reported the molecular weight of lactoperoxidase to be 93,000—the sedimentation constant, $S_{20} = 5.37 \times 10^{-13}$ sec.$^{-1}$; the diffusion constant, $D_{20} = 5.95 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. The partial specific volume was $V = 0.764$ cc/g, and the fractional ratio, $f/f_o = 1.18$.

By light-scattering measurements, Polis and Shmukler *(115)* determined the molecular weight of lactoperoxidase to be 79,000–84,000, which is in good agreement with the value of 81,000 calculated from the iron content, assuming 1 atom of Fe per molecule.

Theorell and Åkeson *(137)* investigated the chemical reactions of the enzyme with $Na_2S_2O_4$, fluoride, and cyanide and H_2O_2, and followed the chemical changes

spectroscopically. They observed two complexes with H_2O_2, one red and the other brown, whose formation was dependent upon the concentration of H_2O_2. Both compounds were unstable and reverted to the enzyme on standing. Chance *(25)* observed a primary complex which was a precursor to the red complex and that the enzyme was also capable of complexing in the same manner with methyl or ethyl hydrogen peroxide. Theorell and Paul *(138)* made further investigations of the fluoride and phosphate complexes involving the sixth coordinate position on the ferric atom in the hemin. Morrell *(95)* further investigated the light absorption of lactoperoxidase and its carbon monoxide-, cyanide-, fluoride-, and pyridine-haemochromogen derivatives and concluded that the prosthetic group was a hematin closely related to protohematin. However, Morrison and Hamilton *(100)* from spectroscopic evidence conclude that it is neither protohemin, hematohemin, nor the hemins a.

Denaturation studies were performed by Polis and Shmukler *(115)* by following the changes in spectra and activity under the influence of acid, heat, and photo-oxidation. The color change is from green to red under the influence of acid and heat. Photo-oxidative denaturation is probably not a true denaturation, since oxidative changes are produced in the aromatic groups of the enzyme.

Electrophoretic analysis of Lactoperoxidase A in phosphate buffer, ionic strength 0.1, indicates an isoelectric point of 6.9. Decreasing the ionic strength shifts it to 7.6 at 0.05 μ and, at 0.01 μ, heterogeneity develops, yielding a slow component isoelectric at 8.0 and a fast component isoelectric at 9.2. In veronal buffer ionic strength 0.1, its isoelectric point is 8.05. For Lactoperoxidase B, they observed an isoelectric point of 6.86 in phosphate buffer, 0.1 μ, but at other pH values it differs in electrophoretic behavior from the A form of the enzyme. Some interesting work remains to be done in this area to explain this behavior, which is common to a number of proteins.

The concentration of lactoperoxidase present in skimmilk calculated from Polis and Shmukler data is 30.3 mg/liter.

ALKALINE PHOSPHATASE

Since Demuth *(32)* first reported the presence of a hexosephosphatase in milk, other investigators *(153)* have observed phosphatase in milk, but it remained for Graham and Kay *(51)* to establish that it was a native component of the milk system and not of bacterial origin. Since that time, considerable work has been done on the use of the enzyme as a measure of the efficiency of pasteurization *(79, 127, 129)*. The need for purified preparations of the enzyme was recognized *(93)*, and Hansson, Solberg, and Sjostrom *(59, 129)* prepared phosphatase concentrates from washed raw cream and Zittle and DellaMonica *(156, 157)* concentrated it from skimmilk. While alkaline phosphatase has not been prepared in crystalline form, Morton *(101, 102)* has prepared a highly purified enzyme (11,000-fold) which electrophoretic evidence suggests is homogeneous and which possesses only phosphomonoesterase activity without the diesterase and pyrophosphatase of the less pure preparations.

Chemical analysis of Morton's *(105)* alkaline phosphatase preparation indicates that it is a colorless, unconjugated protein substantially free of phosphorus ($< 0.1\%$), carbohydrates ($< 2\%$), and nucleotides or related compounds which absorb light at 260 mμ. Its total nitrogen content was found to be 16.2% and its tyrosine and tryptophane contents 27 and 13 moles/10^5 g., respectively. The absorption spectra of the purified alkaline phosphatase show a single maximum at 280 mμ.

There is considerable variability in the alkaline phosphatase content of normal milk *(57, 101)*, and figures are not yet available in the form of mg/liter, but only in activity units/ml, which depend upon the method of measurement. However, the relative distribution among the various milk fractions is established: Approximately 30–40% of the enzyme is concentrated in the cream, where it is absorbed on the fat globule, and the balance is dispersed throughout the skimmilk, probably in the lipoprotein particles. High-speed centrifugation of the skimmilk at 14,000 g. for 30 min. yields a sedimented casein which has very little activity and a supernatant serum which contains most of the enzyme. Recentrifugation of the serum for an additional 2 hr. and 30 min., however, does tend to concentrate it in the precipitate. Churning the cream results in the transfer of the enzyme from the fat globule to the buttermilk.

Heat inactivation of the phosphatases in milk and other dairy products has been the subject of a number of investigations, including those of Hahn and Tracy *(58)*, Hening and Dahlberg *(63)*, Van Bever *(141)*, Hetrick and Tracy *(65)*, and Sanders and Sager *(124)*. These are very useful in the control of pasteurization procedures. As yet, no information is available upon the heat inactivation of the purified enzyme.

OTHER PHOSPHATASE

While phosphodiesterase has not been demonstrated in milk whey with ribonucleic acid as a substrate, partially purified alkaline phosphatase preparations demonstrate some diesterase activity *(157)*. Further purification eliminates this activity *(102)* and demonstrates that there is either a phosphodiesterase in milk or, in some manner, the process of purification has inactivated this function of the enzyme.

Two pyrophosphatases have been reported by Guittonneau, Chevalier, and Jarrouse *(54)* to be present in milk at low concentrations, absent from skimmilk, present in proportion to the fat content in cream, and present in largest amounts in buttermilk and butter serum. One of the enzymes possesses an optimum pH of 4.2 and maintains its activity after heating at 73° C. for 50 min., while the other possesses an optimum pH of 7.6–7.8 and is destroyed by heating to 63° C. for 20 min. It has not been established as yet that these enzymes are native to milk.

Kay and Mullen *(79, 106, 107, 108)* have investigated an acid phosphomonoesterase which is present in milk in low concentrations. Its optimum pH is 4.1, with phenylphosphate as the substrate. This enzyme is less stable than the alka-

line phosphatase when exposed to sunlight or ultraviolet radiation, but is much more heat-resistant, requiring 5 min. at 96° C. for complete destruction. Jacquet and Saingt *(70)* have reported two acid phosphomonoesterases. Their optimum pH values with sodium β-glycerophosphate as a substrate were pH 3.5–4.0 and 5.5–5.8. Their sources have not been conclusively demonstrated.

TRYPSIN INHIBITOR

In 1950, Laskowski and Laskowski *(82)* found a trypsin inhibitor in bovine colostrum which was present in relatively large amounts on the first day after parturition and decreased until, in ordinary cream, its detection was possible only after considerable concentration *(83)*. Assuming that this inhibitor possessed solubilities similar to the trypsin inhibitor prepared from pancreas, these same investigators *(83)* crystallized the trypsin-trypsin inhibitor complex and, finally, the trypsin inhibitor.

The ultraviolet absorption spectra *(80)* of the inhibitor in 0.1 *N* HCl and 0.1 NaOH show a striking difference, characteristic of a substance with a large tyrosine-tryptophane ratio. The absorption maximum shifts from 277 mμ in acid to 295 mμ in alkali. The tyrosine and tryptophane contents *(84)* are 4.94 and 0.39%, respectively, for the inhibitor, as calculated from spectral data, and 6.43 and 3.02% for the trypsin-trypsin inhibitor complex. These last figures agree well with those calculated from inactivation data, 6.62 and 2.93%.

The activity of the crystalline material *(83, 84)* indicated that 1 γ of the colostrum inhibitor inactivates 2.3 γ of trypsin.

The molecular weight of the trypsin-trypsin inhibitor *(84)* complex by light-scattering methods is 89,000. As pointed out by these investigators, this high value may be due to a high molecular weight impurity but, if not, it corresponds to a trimer containing three molecules of trypsin and three molecules of inhibitor. This is in agreement with the activity of tyrosine and tryptophane.

Electrophoretic studies of the inhibitor *(84)* showed a small contaminant at all pH values studied (3.8–5.5) and indicated an isoelectric point of 4.2. The trypsin-trypsin inhibitor complex was electrophoretically homogeneous at all pH values from 4.85 to 9.0 and was isoelectric at pH 7.2. At pH values below 4.85, the complex was electrophoretically heterogeneous, indicating its instability at low pH values.

LIPASES AND ESTERASES

These two groups of enzymes are discussed together, not because the words are synonymous, but because the literature covering them is inseparable. Probably the first report of an esterase, monobutyrinase, in milk was made by Marfan *(88, 89)* in 1901. The first lipase was found in milk by Moro *(97)*, with olive oil as a substrate. Friedjung and Hecht *(48)* proved that monobutyrinase was a native enzyme of milk, by retarding bacterial action with thymol. While this preservative was adequate for esterases, it was not sufficient for lipases of natural fats, since much longer times of incubation were needed. It remained for Rice and Markley *(118)* to prove to the satisfaction of most investigators that lipase

was a native enzyme of milk, by using as a substrate cream saturated with sucrose to prevent bacterial growth. Since then, a large amount of work has been done investigating the phenomena of lipolysis and a number of different enzymes have been postulated on the basis of the effect of substrate, pH, formaldehyde, etc. upon their activity. Mattick and Kay *(90)* and Peterson, Johnson, and Price *(113)* observed a tributyrinase with a pH optimum at 37° C. of 8.5, and the later investigators suggest two enzymes on the basis of stability. Gould *(50)* showed that the lipases in milk hydrolyzed butterfat, cottonseed oil, margarine, hydrogenated cooking fat, corn oil, linseed oil, olive oil, castor oil, coconut oil and lard. Kelly *(80)* showed that they could hydrolyze tricaproin, triacetin, tripalmitin, ethyl oleate, and diacetin with the rate of hydrolysis of the true fats varying inversely with the chain length. Greenbank *(53)* has observed that milk is capable of hydrolyzing α-naphthyl esters. Forster *et al. (44)* employed short-chain fatty acid esters of 2 naphthol-6-sulfonic acid to determine the esterase content of milk. By investigating the relative lipolytic activity toward various substrates (milk fat, Tween-20, tributyrin, and methyl butyrate) in different milk samples, and differential inactivation of these enzyme systems by various agents (aging, heat, light, H_2O_2, copper, acid, alkali, and formaldehyde), Frankel and Tarassuk *(45, 46, 47)* obtained additional evidence of the presence of multiple lipolytic enzymes in milk. Herrington *(64)*, Dormer and Widmer *(39)*, and Gould *(49)* postulate that spontaneously active lipolytic milks contain a different enzyme system from those that require activation by agitation or homogenization, since they are different in their response to various agents, including formaldehyde. Tarassuk and Frankel *(133, 134)* further investigated these enzyme systems and suggest the names "membrane lipase" and "plasma lipase" for the spontaneously active lipase and the lipase of normal milk, respectively. These names were based on their observations that the spontaneously active lipase was irreversibly concentrated in the fat-globule membrane by rapidly cooling to 5° C., while the lipase of normal milk was sedimented with the casein or coagulated with the rennet curd from skimmilk. Dunkley and Smith *(40)* compared tributyrinase and milk-fat lipase of milk and found them to have similar optimum pH values at 10° C., and to possess high correlation coefficients with regard to activity. Schwartz, Gould, and Harper *(127)* characterized the enzymes on the basis of the effect of pH, substrate concentration and formalin concentration, and concluded that there were three different alkaline milk-fat lipases with pH optimum at 7.0, 7.5 to 7.9, and 8.5 at 32° C. Albrecht and Jaynes *(2)*, investigating the enzymes in a similar manner, deduced four acid tributyrinase systems: two with an optimum pH of 5.4 and two at pH 6.3, based upon the formaldehyde sensitivity and substrate specificity. Also, an esterase with a pH range of 5.7–6.0 was observed.

In spite of all the work done on the lipase systems of milk and the valuable practical information secured, no completely convincing conclusions can be reached with regard to the number of lipase systems involved, nor the mechanism of their action, until highly purified preparations have been made. It is sur-

prising that, in view of the great interest in the dairy field in lipolysis, only two attempts at such preparations have been reported. Tarassuk and Frankel *(133)* have reported that they are attempting a concentration and isolation of lipase, based upon its concentration in the foam and its stability to surface denaturation. More recently, these same authors *(134)* have suggested the use of the fat-globule membrane material as a source for the concentration and purification of the spontaneously active lipase and the casein fraction for the lipase of normal milk. Harper, Gould, and Badami *(61)* reported the separation of the two alkaline milk-fat lipases by supercentrifugation with the one lipase with optimum pH 8.6 in the sediment, and the other with optimum pH at 7.0 in the supernatant. This work should be continued.

The information available in the literature concerning the chemical behavior and denaturation of lipase is confusing, due to our lack of knowledge as to which lipase systems are involved. While considerable practical knowledge is available, basic knowledge can be obtained only when the lipase systems are better defined.

ANTIBACTERIAL FACTORS

The antibacterial properties of milk were first observed by Fokker *(43)* in 1890. Following his paper, little was done concerning this phenomenon until a series of papers by Jones and his coworkers *(73, 74, 75, 76, 77)*, in which they noted that the growth of streptococci, especially *Streptococcus pyrogenes*, was inhibited by milk and rennet whey. The inhibitory substance would not pass through a semipermeable membrane and was destroyed by trypsin. Based upon these properties, Jones and Simms *(77)* were able to prepare a dried material which was 200 times as active as the original milk. They called this material lactenin. Wilson and Rosenblum *(150, 151, 152)* concluded from their experiments that lactenin was neither xanthine oxidase nor lysozyme. They also showed that the lactenin was inactive under anaerobic conditions and in the presence of -SH compounds. Auclair and Hirsch *(10)* confirmed the results of Wilson and Rosenblum by comparing the action of raw milk and a lysozyme preparation upon *S. pyrogenes* and *Micrococcus lysodeikticus*. They also concluded that there are two lactenins in milk: L_1, occurring mainly in colostrum and most stable to heat at pH 6.0–6.5, with ~90% being destroyed at 68° C. for 20 min.; and L_2, occurring mainly in milk and most stable at pH 7.0, with 95% being destroyed at 74° C. for 20 min. They were shown also to be synergistic in their antibacterial action. Auclair *(7)* and Berridge *(18)* investigated the action of the lactenins on a number of organisms with the following results: Inhibited: *Bacillus subtilis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus lactis* (in milk), and *Lactobacillus helveticus* (slightly); not affected: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Streptococcus faecalis*.

Flipse, Patton, and Almquist *(42)* found that raw skimmilk and a lactenin preparation were very toxic to bovine spermatozoa. However, the preparation was not pure and possessed both amylase and protease activity.

Auclair and Berridge *(8, 9)* attempted a fractionation and isolation of the two lactenins and were able to partially fractionate and to concentrate the lactenins. Electrophoretic patterns of fractions rich in each of the lactenins indicate considerable heterogeneity at pH 7.0 in 0.05 *M* phosphate buffer. Purer fractions are needed for more satisfactory characterization of these materials.

Hobbs *(67)*, Morris *(98)*, and Morris and Edwards *(99)* found an inhibitor in raw whole milk which in a few hours reduces the number of bacteria to a very low number in the case of *E. coli*, *S. aureus*, *Staphylococcus albus*, *Staphylococcus citreus*, *S. lactis*, and *S. agalactiae*. Since it is effective with organisms which are not acted upon by lactenin, Berridge *(18)* does not consider it to be lactenin. Morris and Edwards *(99)* observed microscopically the death of organisms and concluded that it was not due to agglutination or ordinary lysis but that the organism lost its ability to refract light. It has not been proved to be a protein, nor has any successful effort been made to isolate it, but it is inactivated by heating to 55° C. for one-half hour. Much more work needs to be done.

The presence of a lysozyme in bovine milk as a native ingredient is questionable. Auclair and Hirsch's *(10)* results with *M. lysodeikticus* are uncertain. If present, it must be in very low concentration. Seleste *(128)* observed small amounts in three out of ten cows tested.

AMYLASE

Amylase was first definitely observed in bovine milk by Zaitschek *(155)*, but it remained for Richardson and Hankison *(119)* to establish that it was a native protein of bovine milk. Based upon the work of others in the field of plants and animal amylases, they made a distinction between α-amylase, the liquefying and dextrinizing enzyme, and β-amylase, the saccharifying enzyme, and their work demonstrated that α-amylase was definitely present and that the milk possessed a small amount of saccharifying action suggesting the presence of β-amylase. The effect of heat on the amylase actions indicates that the dextrinizing action is adversely affected by heating for 30 min. at 50° C., while the saccharifying action is not inhibited until the temperature reaches 70° C. for 30 min., or 90° C. flash. Chrzaszcz and Goralowna *(29)* found that colostrum and milk from diseased udders showed greater amylase activity than normal milk. They also found that the amylase seems to concentrate in the cream, and that most of it is precipitated with the casein, although some remains in the whey. However, Guy and Jenness *(55, 56)* found the α-amylase to be concentrated in the skimmilk and whey and prepared a highly concentrated α-amylase system from the protein fraction precipitated from whey at 43% saturation with $(NH_4)_2SO_4$, which is capable of liquefying, dextrinizing, and saccharifying starch. They also demonstrated that a single enzyme appeared to be responsible for all the activity, by obtaining a single pH optimum for the properties of the enzyme at pH 7.4 and 34° C. and by securing a linear plot for the velocity vs. velocity/substrate concentration for its saccharifying action. However, some of their results suggest

the presence of small amounts of β-amylase in some milks. Calcium and chloride ions are shown to be essential for the α-amylase activity.

PROTEASES

The presence of a proteolytic enzyme in milk was first reported in 1897 by Babcock and Russell *(14)*. While there were some questions as to the origin of this protease still being expressed in 1917 *(136)*, their evidence reported in 1898 *(15)*, indicating that it is a native constituent of milk, is as convincing as any reported since. They employed freshly drawn samples from healthy cows and immediately treated them with chloroform in concentrations sufficient to prevent bacterial growth in the samples.

Warner and Polis *(145)* found that almost all of the proteolytic activity of the milk is precipitated with the casein, and they developed a procedure for its concentration. A 150-fold increase in activity was obtained. The protease is resistant to heat, requiring 80° C. for 10 min. in borax buffer for its destruction. Its optimum activity is achieved at pH 9.2 in borax buffer, and it is similar to trypsin in action. In fractionating casein with alcohol and urea, Hipp, Groves, Custer, and McMeekin *(66)* found that the protease was concentrated in the α-casein fraction, but could be removed by fractional precipitation with alcohol at pH 6.0. Kadaba and Herreid *(78)*, in storage studies of sterile milk, observed what appears to be a reactivation of the HTST heat inactivation of the protease and that this reactivated enzyme is specific for β-casein. Further purification and characterization of this enzyme are needed. Wohlgemuth and Strech *(154)* reported the presence of a peptidase in bovine milk which split glycyl-trptophane, but Heiduschka and Komm *(62)* were unable to confirm their results with fresh milk. This enzyme probably is not a native enzyme of milk.

CATALASE

The presence of catalase in bovine milk was reported in 1911, independently, by Spindler *(131)* and Kooper *(81)*. Since then, it was observed that increased bacterial or leucocyte counts are always followed by increased catalase content. This raised some question as to the occurrence of catalase in normal milk; however, Reid *(117)* found catalase in aseptically drawn milk from healthy cows. He also observed that colostrum had a higher concentration of the enzyme than does the milk, yet the catalytic activity is precipitated with the casein *(91)*. McMeekin *(91)* reports that the enzyme is concentrated in the cream and separator slime. A number of investigators *(24, 41, 91)* have investigated the heat denaturation of catalase in milk with different results, probably due to differences in the milk or in the techniques investigated.

OTHER ENZYMES

A number of other enzymes have been reported in milk: An enzyme system which breaks down lactose into CO_2, lactic acid, ethyl alcohol, and acetic acid, by Stoklosa *(132)* in 1904, which Vandevelde *(143)* showed did not form glucose

and galactose; salolase by Vandevelde *(144)* in 1907; aldolase, which reversibly splits fructose 1,6–diphosphate into dihydroxyacetone and phosphoglyceric aldehyde, by Polis and Shmukler *(114)*; and carbonic anhydrase which dehydrates carbonic acid and hydrates carbon dioxide, and rhodonase which converts cyanides to thiocyanates, by Alfonso and Bertran *(3)*.

Polis and Shmukler *(114)* found that their aldolase was concentrated in the cream. They were also able to achieve a fifty-fold concentration by removing the casein with 1.5 *M* $(NH_4)_2SO_4$ and subsequent salt fractionation of the whey at 3° C., with the aldolase being concentrated in the 2.4 *M* to 2.8 *M* salt. Aldolase is rapidly inactivated by heat, being completely inactivated in milk by 20 min. heating at 45° C.

Considerably more work is needed on all of these enzymes, even to the extent of proving more conclusively that they actually are native constituents of normal milk.

A RED PROTEIN

In 1939, Sorensen and Sorensen *(130)* reported the separation of a red protein from milk, but made no further effort to identify the material. Later, in preparing lactoperoxidase, Theorell and Åkeson *(137)* obtained a red protein also, which they suggested might not be native to milk but a break-down product of peroxidase, since its concentration increases during the fractionation of peroxidase. Morton *(102)*, in his procedure for purifying the alkaline phosphatase, noted a red protein with little phosphatase activity, which was obtained by 63% saturation of the serum with $(NH_4)_2SO_4$ after treatment of buttermilk with *n*-butanol, ether, and acetone. The color was completely reduced with sodium dithionite and resembled xanthine oxidase. While the fraction contained some xanthine oxidase activity, Morton considered that its intense red color could not be due to xanthine oxidase alone.

Polis and Shmukler *(115)* confirmed Theorell and Åkeson's observations and isolated the red protein which is almost electrophoretically homogeneous with a mobility of 2.2 in veronal-NaCl buffer, pH 8.4, and 0.1 ionic strength. The absorbancy ratios from its adsorption spectra were 280:250 $m\mu = 1.78$; 412:280 $m\mu = 0.024$, and 412:310 $m\mu = 0.15$. These investigators also supported the view of Theorell and Åkeson that this might be a break-down product of lactoperoxidase. Obviously, more work needs to be done before a definite conclusion can be reached.

δ-CASEIN

Cherbuliez and his coworkers, in a series of papers *(26, 27, 28)*, have obtained a casein fraction which they designated as δ-casein, which amounts to about 4% of the total casein fraction. Its electrophoretic mobility in veronal buffer pH 8.7 and ionic strength 0.1 is less than that of γ-casein. Chemical analyses indicate that it contains 0.55% phosphorus, 0.10% sulfur, 4.12% methionine, and $< 0.2\%$ tryptophane. These investigators have compared its chemical and physical properties with those of Hammarsten's proteose *(28)*, obtained in the formation

of paracasein, and have found them to be practically the same; therefore, they concluded that they are identical and that, therefore, the Hammarsten proteose is not a degradation product of the casein. The reverse interpretation is a possibility, although the concentrations of Hammersten's proteose and δ-casein are approximately the same.

OTHER PROTEINS

A number of other minor proteins, or minor protein fractions, which are not known to be enzymes but are recognized by other properties have been reported. Dills and Nelson *(34)* have prepared a protein fraction from bovine milk which contains 15% nitrogen and 0.19% copper in a nondialyzable form at pH 6.5, but in a dialyzable form at pH 3.5. Due to the lack of sufficient material they were unable to characterize the fraction further or to determine its homogeneity. However, it does compare favorably with other copper-containing proteins. Tests for ascorbic acid oxidase and polyphenolase activity were negative.

Aschaffenburg and Ogston *(5)* investigated a protein fraction obtained from milk which appears to possess the surface-active components of milk serum after the removal of the casein and heat-coagulable proteins, which they have called σ-proteose. This material was obtained by repeated precipitation from the serum by half-saturation with $(NH_4)_2SO_4$, dialysis of the precipitate against water, and lyophilization. It has the following chemical analysis: C, 49.85%; H, 7.0%; N, 13.95%; P, 0.68%; S, 0.72%; Ca, 0.6%. From solubility studies, it is isoelectric at pH 4.5. Electrophoretically, it possesses three components at pH 8.0, $\mu = 0.2$ in phosphate buffer. In the ultracentrifuge in 0.2 *M* NaCl, 0.017 *M* Na_2HPO_4, and 0.017 KH_2PO_4 pH 6.8, it appears to possess two distinct components with $S_{20} = 0.96 \times 11^{-13}$ sec.$^{-1}$ and 2.75×10^{-13} sec.$^{-1}$. They account for 49 and 11% of the total refractive material, respectively. The balance may be too polydispersed, or too high or low molecular weight, to show. With regard to the question as to whether this protein is native to milk or a result of the heating necessary to coagulate the coagulable proteins, the same problems are involved as with the "proteose-peptone" fraction.

Weinstein, Duncan, and Trout *(146, 147)*, by a method very similar to that of Aschaffenburg, prepared a protein fraction which, while it had surface-active properties, was also found to contain the precursor to a solar-activated flavor. It also differed somewhat in its physical and chemical properties from σ-proteose. Its chemical analysis is as follows: C, 44.2%; H, 6.35%; N, 10.00%; P, 0.27%; S, 1.30%. The isoelectric zone for this material is pH 4.1–4.3. Electrophoretic studies at a number of different pH values, buffers, and ionic strengths indicate that it consists of at least two electrophoretic components.

LIPOPROTEINS AND THE FAT-GLOBULE-MEMBRANE PROTEINS

While we no longer consider the fat-globule-membrane protein to be a single species, a brief summary of the progress of our knowledge of this group of proteins is essential to a review of the minor proteins of milk. Since Ascher-

son *(6)* formulated his haptogen membrane theory concerning the composition of the fat-globule-membrane, it has been recognized to consist in part of protein. Information regarding its over-all composition and structure was secured by Palmer and his coworkers in a series of papers *(72, 108, 110, 111, 112, 122, 135, 148, 149)*. The washing and churning method which they employed for the isolation of the membrane material is still the most effective procedure. More recently, information concerning the amino acid composition, and electrophoretic and ultracentrifuged properties, has been secured by Brunner and his coworkers *(21, 22, 23)*, and the amino acid compositions by Hare, Schwartz, and Weese *(60)*. Morton *(102, 104)* and Zittle *et al. (158)* investigated the enzyme content of the membrane and found, as noted earlier in the review: alkaline phosphatase, xanthine oxidase, nucleic acid, and cytochrome-c. Morton *(102, 104)* has proposed that, in addition to the fat-globule membrane lipoproteins, there are lipoprotein complexes of similar composition which he calls "microsomes", about 100 mμ in diameter, not only absorbed on the fat-globule but distributed throughout the aqueous phase. The make-up of these lipoprotein particles will undoubtedly be an interesting subject of additional research.

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ELECTROPHORETIC PROPERTIES OF CASEIN FROM STERILIZED MILK STORED AT DIFFERENT TEMPERATURES [1]

LALITHA MURTHY,[2] E. O. HERREID, AND R. McL. WHITNEY
Department of Food Technology, University of Illinois, Urbana

SUMMARY

High-temperature short-time sterilization of milk did not hydrolyze the proteins but it denatured some serum proteins which were precipitated with the casein, and either moved at the same mobility at pH 8.7 as α-casein, or was associated with it and increased the area under the peak. A split in α-casein appeared in some cases during storage. Preheating, order of homogenization and storage temperatures, and time affected the relative areas of α- and β-casein from sterile milk. The amount of α-casein plus denatured serum proteins did not change greatly during storage for milk preheated at either 140 or 180° F., and homogenized either before or after sterilization. The mobilities varied for α-casein from milk preheated to 180° F., homogenized either before or after sterilization; but those for milk preheated to 140° F. and otherwise treated similarly were unchanged. The amount of β-casein from sterile milks varied with the preheating and storage temperatures and homogenization. The breakdown of β-casein to a new component, X, with a mobility near that of γ-casein, and more noncasein and nonprotein nitrogen in milk preheated to 140° F., was greatest at 100, intermediate at 70, and least at 40° F. storage. This breakdown occurred, also, in milk preheated to 180° F., but was insufficient to increase noncasein and nonprotein nitrogen. The breakdown of β-casein may be caused by a protease.

Heating fluid milk is the only commercial method at present for preserving its keeping quality and food value. The intensity of heating depends upon the product for which milk is intended. In recent years, the trend in the dairy industry has been to use continuous methods for processing milk and milk products with short or practically no holding time, thus preserving, insofar as possible, its nutritional properties and palatability *(3)*. During processing, the labile milk proteins are exposed to different temperatures which affect the properties of the final product. More information is needed on the effects on proteins of sterilizing milk and storing it at different temperatures for extended periods.

It has been observed that there are no significant changes in the nitrogen distribution of milk during pasteurization *(11, 15)*. The heat-denatured serum proteins are precipitated during sterilization of evaporated milk. The evaporating process causes small changes in the nitrogen distribution of skimmilk *(11)*. No change was observed in the nonprotein nitrogen *(21)* in milk pasteurized either by the holder or by the high-temperature-short-time methods.

Electrophoretic analysis of heated milk has shown that casein is altered in evaporated milk *(16)*. Analysis by paper electrophoresis showed that heating, boiling, and pasteurizing milk decreased the proportions of the first two components and increased the third component. A third component appeared in

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milk heated by a steam injection method called uperization *(1)*. Changes were observed in the electrophoretic pattern of skimmilk when heated to 65, 75, and 100° F. for 30 min. *(17)*. When milk is sterilized, there are structural changes in α-casein and β-lactoglobulin affecting their mobility at pH 8.7 *(19)*, and complex formation between α-casein and lactose *(13)*. The extent of these changes depends on the heat treatment and the concentration of milk *(13)*. The whey proteins are denatured partially or completely, depending upon the time and temperature *(8)*. However, most of the work cited in the literature was with skimmilk, processed under laboratory conditions. Investigators *(4, 10, 19, 20)* have studied the effects of heating some milk products in a Mallory small-tube heat exchanger.

No data are available on the electrophoretic properties of proteins in milk processed, commercially, and stored for 5–6 mo. at different temperatures. It was the purpose of this study to determine the nitrogen distribution and electrophoretic properties of casein in whole milk sterilized in a Mallory small-tube heat exchanger *(10)* with practically no holding time.

MATERIALS AND METHODS

Sterilized milk. Milk was obtained from the University farm, clarified at 90–95° F., then standardized to 3.5–3.7% fat. In the first series, two different batches of milk were preheated in a stainless steel vat, the first to 140 and the second to 180° F. It was homogenized at 2,500 p.s.i.g., sterilized at 305 ± 0.25° F., and cooled to 45° F. This milk was collected in a sterile 10-gal. can directly from the Mallory small-tube heat exchanger and bottled aseptically *(3)*. The bottles were divided equally into three lots, labeled, and stored at 40, 70, and 100° F. The original raw and the freshly sterilized milk were analyzed for total nitrogen, noncasein nitrogen, and nonprotein nitrogen. Electrophoretic analyses were made of the caseins prepared from these milks. The stored samples were treated in the same manner at intervals of 4, 8, 12, and 20 or 24 wk.

To eliminate variations due to different milks, and to compare the effects of various preheating temperatures and the effects of homogenization before and after sterilization, mixed raw whole milk was clarified, divided into four batches, and treated as follows: Batches 1 and 2 were preheated to 140 and 180° F., respectively, homogenized at 2,500 p.s.i.g., sterilized at 305 ± 0.25° F., cooled to 45° F., bottled, and stored. Batches 3 and 4 were preheated to 140 and 180° F., respectively, sterilized at 305 ± 0.25° F., homogenized at 153° F., cooled to 45° F., and aseptically bottled. The milks were homogenized after they were sterilized with a special homogenizer assembly, attached to the cooling section of the Mallory small-tube heat exchanger. They were stored at the three temperatures.

The milk samples were warmed to 77° F. and thoroughly mixed before they were analyzed by the following procedures:

Total nitrogen by direct nesslerization of the digested sample. One ml. of milk was diluted to 500 ml. in a volumetric flask with NH_3-free water. A 10-ml.

aliquot of the diluted milk was digested, and the nitrogen content was determined according to the method of Hetrick and Whitney *(5)*. The color developed was measured in a Coleman Model 11 spectrophotometer at 420 mμ.

Noncasein nitrogen. The casein in 10 ml. of milk was precipitated according to Rowland's method *(14)* and removed by filtration through a No. 42 Whatman filter paper. The noncasein nitrogen was determined in 1 ml. of the casein-free filtrate in the same manner as in the total nitrogen determination.

Nonprotein nitrogen. The proteins in 10 ml. of milk were precipitated with 15% trichloroacetic acid according to Rowland's method *(14)* and removed by filtration through a No. 42 Whatman filter paper. One ml. of the filtrate was digested as before and the nonprotein nitrogen content of the sample was determined by direct nesslerization of the digested sample.

TABLE 1

Effect of time and temperature of storage on the nitrogen distribution in sterile milk

Storage *(wk.)*	Total N	Noncasein N	Casein N	Nonprotein N
	(mg. N/100 ml.)			
	Milk preheated to 140° F., homogenized, and sterilized			
Raw	544.3	123.1	421.2	36.1
	Stored at 40° F.			
0	551.1	79.2	471.9	34.2
4	558.8	69.4	486.4	35.0
8	582.5	82.5	500.0	40.7
12	593.0	81.2	511.8	45.2
24	543.5	85.6	457.9	34.9
	Stored at 70° F.			
4	551.1	71.6	479.5	39.2
8	590.6	109.9	480.7	47.3
12	618.2	100.6	517.6	42.7
24	521.1	119.5	401.6	44.7
	Stored at 100° F.			
4	565.1	88.7	476.4	41.3
8	593.1	133.3	459.8	56.9
12	629.4	144.5	484.9	58.5
24	558.9	157.2	401.7	59.6
	Milk preheated to 180° F., homogenized, and sterilized			
Raw	490.4	129.2	361.2	39.5
	Stored at 40° F.			
0	495.1	70.1	425.6	40.4
4	517.8	73.4	444.4	42.3
8	502.8	67.1	435.7	42.1
12	518.4	70.1	448.3	43.1
24	475.9	65.3	410.6	43.5
	Stored at 70° F.			
4	514.5	68.9	445.6	41.2
8	498.2	72.7	425.5	39.5
12	518.4	73.2	445.2	44.6
24	464.9	72.8	392.1	47.3
	Stored at 100° F.			
4	520.7	78.1	442.6	44.6
8	506.5	77.3	429.2	43.1
12	515.6	75.3	440.3	47.4
24	474.1	83.1	391.0	49.2

Preparation of casein. The casein for the electrophoretic study was obtained by the same method as that employed for the noncasein nitrogen determination. It was then suspended in 100 ml. of distilled water in a centrifuge bottle, mechanically stirred for 5 min. and centrifuged at 1,400 r.p.m. for 15 min. The supernatant was discarded. To remove occluded fat, the casein was treated the same way with six solvent washings in this order: 100 ml. acetone, 75 ml. acetone, 100 ml. acetone: ethyl ether mixture (1:1), 75 ml. acetone: ether mixture (1:1), 100 ml. ethyl ether, and 75 ml. ethyl ether. The casein was finally dried at 70–75° F. with an aspirator. The casein samples were stored in bottles in a desiccator under vacuum at 40–44° F. The effect of the solvent treatment on the electrophoretic mobilities of casein was not significant at the 1% level.

Electrophoretic analysis. One per cent casein solutions were prepared by dissolving 0.2 g. of casein in 5 to 10 ml. of sodium veronal buffer of pH 8.7 and an ionic strength of 0.1, then diluting to 20 ml. with the same buffer. The protein solutions were dialyzed against sodium veronal buffer at 39.2° F. for 24 hr. Electrophoresis was done at ice-bath temperatures for 1 hr. with a current of 12.0 Ma in a Perkin-Elmer Tiselius apparatus, Model 38A, equipped with Philpot-Svensson cylindrical-lens system.

Mobilities were calculated from the false boundaries of both the ascending and the descending patterns. The areas under the curve were divided by the method suggested by Tiselius and Kabat *(18)* and measured with a planimeter including the δ and ϵ boundaries. Corrections for the δ and ϵ effects were made as suggested by Longsworth *(9)*. Unless otherwise stated, all experiments were done under similar conditions.

Calculations of the nitrogen distribution within the casein. To calculate the changes in the amounts of the various casein components in milk during storage, the amounts of nitrogen contributed by α-, β-, and X-components of casein to the total casein nitrogen in raw milk and total casein, plus denatured serum-protein nitrogen in sterilized milk stored at different temperatures, were calculated as follows:

Noncasein nitrogen in original raw milk $= x$ mg. per 100 ml.

Noncasein nitrogen in either freshly sterilized or stored milk $= y$ mg. per 100 ml.

Nitrogen due to denatured serum proteins $= (x - y)$ mg. per 100 ml.

Casein nitrogen in original raw milk $= z$ mg. per 100 ml.

Casein nitrogen in either freshly sterilized or stored milk $= z + (x - y)$ mg. per 100 ml.

Per cent relative area of the α-, β-, or X-component of casein on the ascending or the descending side $= A$

α-casein nitrogen in raw milk $= \frac{z \times A}{100}$ mg. per 100 ml.

α-casein nitrogen in sterilized or stored milk $= \left[\frac{z + (x - y)}{100}\right] \times A$ mg. per 100 ml.

TABLE 2

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 140° F., homogenized, and sterilized[a]

Storage (*wk.*)	Casein component	Temperature					
		40° F.		70° F.		100° F.	
		D[b]	A[c]	D[b]	A[c]	D[b]	A[c]
		Mobility (*cm.*^{2} *sec.*$^{-1}$ $v^{-1} \times 10^{-5}$)					
Raw	α			−5.91	−6.73		
	β			−3.33	−4.15		
0	α[d]			−5.85	−6.61		
	β			−3.42	−4.02		
	X				−2.78		
4	α[d]	−6.03	−6.79	−5.97	−6.78	−5.99	−6.94
	β	−3.45	−4.21	−3.33	−4.25	−3.32	−4.21
	X		−2.75		−2.82	−1.72	−2.85
8	α[d]	−6.06	−6.87	−5.77	−6.41	−6.17	−7.00
	β	−3.34	−4.33	−3.20	−3.78	−3.44	−4.48
	X				−2.21	−1.86	−2.97
12	α[d]	−5.95	−6.81	−5.97	−6.83	−5.97	−6.89
	β	−3.45	−4.25	−3.36	−4.21	−3.42	−4.27
	X				−2.95	−1.71	−2.96
24	α[d]	−6.02	−6.84	−5.91	−6.72	−5.80	−6.67
	β	−3.36	−4.16	−3.36	−4.29	−3.48	−4.23
	X		−2.78	−3.10	−2.90	−1.86	−2.96
		Relative area (%)					
Raw	α			74.0	67.7		
	β			26.0	32.3		
0	α[d]			77.7	72.6		
	β			22.3	25.8		
	X				1.6		
4	α[d]	78.6	73.2	82.0	72.2	85.5	75.3
	β	21.4	26.0	18.0	24.6	13.0	17.8
	X		0.8			1.5	6.9
8	α[d]	80.3	73.5	82.8	77.8	89.5	80.9
	β	19.7	26.5	17.2	21.2	8.9	10.7
	X				1.0	1.6	8.4
12	α[d]	80.2	75.8	87.1	80.0	93.3	81.2
	β	19.8	24.2	12.9	15.4	4.3	8.0
	X				4.6	2.4	10.8
24	α[d]	80.6	73.5	87.7	79.3	94.7	88.7
	β	19.4	24.4	9.2	12.7	1.6	4.8
	X		2.1	3.1	8.0	3.7	6.5

[a] Corresponds to milk with the same treatment (Table 1).
[b] Descending.
[c] Ascending.
[d] α-Casein plus denatured serum proteins.

Since the total nitrogen values in stored milk showed an unexplained variation *(12)*, the casein nitrogen contents of the raw milks were used as reference values instead of the individual casein nitrogen values in the stored milk. However, for noncasein nitrogen in sterilized milk the individual values were used.

RESULTS

Four different samples of raw and the corresponding freshly sterilized and stored milks at 40, 70, and 100° F. were analyzed for nitrogen distribution. Since the results for the four different batches of milk were similar, typical data

for one of the batches treated in different ways are given (Table 1). The electrophoretic properties of casein from the same milks used for nitrogen distribution were made in sodium veronal buffer at pH 8.7 and ionic strength 0.1. The results are presented (Tables 2 and 3). The third component in the raw milk is reported as γ-casein, but in the freshly sterilized milk and in that stored, it is designated as component X. Whenever α-casein and denatured serum proteins showed a split, it was reported as α_1, and α_2. The nitrogen distributions for the various casein components in Tables 2 and 3 are given (Table 4).

TABLE 3

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 180° F., homogenized, and sterilized[a]

Storage (*wk.*)	Casein component	Temperature					
		40° F.		70° F.		100° F.	
		D[b]	A[c]	D[b]	A[c]	D[b]	A[c]
		Mobility (*cm.^{2} sec.$^{-1}$ v^{-1} × 10^{-5}*)					
Raw	α			−6.26	−6.91		
	β			−3.54	−4.25		
	γ			−2.24	−2.89		
0	α[d]			−5.73	−6.31		
	β			−3.27	−3.86		
4	α[d]	−6.20	−6.96	−6.49	−6.84	−5.91	−6.78
	β	−3.30	−4.29	−3.42	−4.06	−3.30	−4.17
	X					−1.86	
8	α[d]	−6.12	−6.85	−6.23	−6.91	−6.23	−6.86
	β	−3.40	−4.19	−3.29	−4.25	−3.51	−4.19
	X						−2.95
12	α[d]	−5.91	−6.67	−6.26	−7.13	−6.31	−6.84
	β	−3.28	−4.10	−3.48	−4.29	−3.50	−4.14
	X						−2.53
24	α[d]	−5.94	−6.76	−6.26	−6.89	−6.15	−6.84
	β	−3.53	−4.16	−3.45	−4.13	−3.56	−4.25
	X						−2.69
		Relative area (*%*)					
Raw	α			75.5	66.3		
	β			23.5	29.8		
	X			1.5	3.9		
0	α[d]			77.5	73.6		
	β			22.5	26.4		
4	α[d]	80.8	76.2	82.3	78.7	80.7	76.0
	β	19.2	23.8	17.7	21.3	18.0	24.0
	X					1.3	
8	α[d]	83.0	78.8	82.6	79.6	83.0	78.5
	β	17.0	21.2	17.4	20.4	16.8	20.2
	X						1.3
12	α[d]	83.6	76.9	84.5	80.6	86.9	79.8
	β	16.4	23.0	15.5	19.4	13.1	18.2
	X						2.0
24	α[d]	83.5	79.2	86.2	79.2	84.6	80.5
	β	16.5	20.8	13.8	20.8	15.4	17.6
	X						1.9

[a] Corresponds to milk with same treatment (Table 1).
[b] Descending.
[c] Ascending.
[d] α-Casein plus denatured serum proteins.

TABLE 4

Effect of time and temperature of storage on the α-, β-, and X-components of the casein nitrogen content of sterile milk[a]

Storage (wk.)	Temperature								
	40° F.			70° F.			100° F.		
	α[b]	β	X	α[b]	β	X	α[b]	β	X
	(mg. N/100 ml.)								
Calculated from areas of descending electrophoretic patterns									
Milk preheated to 140° F., homogenized, and sterilized									
Raw				311.7	109.5				
0				361.4	103.7				
4	381.3	93.6		386.8	85.9		389.5	59.3	6.8
8	370.8	91.0		359.7	74.7		368.8	36.6	6.6
12	371.4	91.7		386.5	57.2		373.0	17.2	9.6
24	370.2	88.5		372.5	39.1		387.1	6.2	14.3
Milk preheated to 180° F., homogenized, and sterilized									
Raw				270.9	84.8				
0				325.7	94.6				
4	336.9	80.1		346.9	74.6		332.7	74.2	
8	351.4	72.0		345.0	72.7		343.7	69.4	
12	351.4	68.9		352.5	64.7		360.7	54.5	
24	354.9	70.1		360.0	57.6		344.6	62.7	
Calculated from areas of descending electrophoretic patterns									
Milk preheated to 140° F., homogenized, and sterilized									
Raw				285.2	136.0				
0				337.6	120.0	7.4			
4	347.6	123.4	3.9	341.3	116.0	15.1	343.1	81.4	31.4
8	339.4	122.4		338.0	92.1	4.3	332.5	44.0	34.5
12	351.0	112.1		354.9	68.3	20.5	324.6	32.0	43.6
24	337.1	111.9	9.7	336.9	54.0	33.9	343.3	18.6	25.2
Milk preheated to 180° F., homogenized, and sterilized									
Raw				239.5	107.9	14.1			
0				309.3	111.0				
4	317.8	99.2		331.7	89.0		313.0	99.0	
8	333.6	89.8		332.5	85.2		324.3	83.4	5.4
12	323.2	97.1		336.2	80.9		331.2	75.5	8.3
24	336.7	88.4		330.4	86.9		327.8	71.7	7.8

[a] Data taken from Tables 1, 2, and 3.
[b] α-Casein includes some denatured serum proteins.

The effects of preheating milk to 140 and 180° F., and of homogenization before and after sterilization on the electrophoretic properties of casein from raw, freshly sterilized milks and those which were stored at 40, 70, and 100° F., are reported (Tables 5 to 8). The electrophoretic patterns of these samples only are shown (Figures 1 to 5), because the patterns of the others were similar.

DISCUSSION

The nitrogen values determined by the direct nesslerization of the digested sample showed an unexplained variability with storage (Table 1). Murthy and Herreid *(12)* compared the total nitrogen in sterilized milk stored at 70° F. by both the macro-Kjeldahl and micro-method, and reported that there were variable factors in the direct nesslerization method for stored milks that need further study. Also, recently, Jamil *(7)* reported that the nesslerization method gave higher values for certain foods than did the macro-Kjeldahl method.

TABLE 5

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 140° F., homogenized, and sterilized

Storage (*wk.*)	Casein component	Temperature					
		40° F.		70° F.		100° F.	
		D[b]	A[c]	D[b]	A[c]	D[b]	A[c]
		Mobility (*cm.*^{2} *sec.*$^{-1}$ $v^{-1} \times 10^{-5}$)					
Raw	α			−6.00	−6.86		
	β			−3.14	−4.28		
	γ				−3.09		
0	α^1			−5.67	−6.23		
	α^a			−6.35	−6.69		
	α^2			−3.45	−4.19		
	β						
4	α^1		−6.61				
	α^a	−6.03		−6.20	−6.96	−6.14	−7.02
	α^2		−7.06				
	β	−3.50	−4.37	−3.30	−4.29	−3.45	−4.33
	X	−2.47	−3.39		−3.01	−1.99	−3.15
8	α^1				−6.32		
	α^a	−6.00	−6.70	−5.85		−6.08	−6.79
	α^2				−6.85		
	β	−3.49	−4.25	−3.22	−4.21	−3.28	−4.21
	X		−2.80	−1.93	−2.93	−1.93	−3.04
12	α^1		−6.26				−6.54
	α^a	−5.86		−5.86	−6.72	−5.91	
	α^2		−6.78				−7.18
	β	−3.22	−4.14	−3.33	−4.19		
	X		−2.87	−1.95	−2.81	−1.78	−2.99
20	α^a	−5.91	−6.90	−5.74	−6.84	−6.09	−6.83
	β	−3.30	−4.28	−3.42	−4.29		
	X		−2.90	−1.91	−2.90	−1.94	−2.85
		Relative area (*%*)					
Raw	α			73.9	68.7		
	β			26.1	29.6		
	γ				1.7		
0	α^1			43.8	32.0		
	α^a						
	α^2			34.7	40.7		
	β			21.5	27.3		
4	α^1		29.2				
	α^a	77.5		81.3	73.6	88.8	80.3
	α^2		43.4				
	β	20.9	24.8	18.7	22.8	7.4	12.9
	X	1.6	2.6		3.6	3.8	6.8
8	α^1				27.8		
	α^a	81.4	71.5	83.9		91.8	86.6
	α^2				50.1		
	β	18.6	26.4	13.2	17.4	3.3	4.6
	X		2.1	2.9	4.7	4.9	8.8
12	α^1		38.3				54.3
	α^a	80.5		88.0	81.8	92.6	
	α^2		36.4				31.5
	β	19.5	22.8	7.4	9.1		
	X		2.5	4.6	9.1	7.4	14.2
20	α^a	83.5	78.2	87.5	84.8	90.8	93.4
	β	16.5	20.1	3.5	5 5		
	X		1.7	9.0	9.7	9.2	6.6

[a] α-Casein plus denatured serum proteins.
[b] Descending.
[c] Ascending.

Sterilization at 305° F. denatured some serum proteins (Table 1), which were precipitated with the casein and either moved at approximately the same mobility in sodium veronal buffer of pH 8.7 and an ionic strength of 0.1 or were associated with the α-casein. There was a real increase in the α-casein nitrogen (Table 4) which indicated that some of the denatured serum proteins were in the α-casein component, and no other new component appeared in the electrophoretic pattern of casein (Figure 1), except in the casein from milk preheated to 140° F., homogenized, and sterilized. Slatter and Van Winkle *(17)* reported that heating milk for 30 min. at 65, 75, and 85° C. increased the concentration of protein migrating with α-casein in a buffer of 6.6 and an ionic strength of 0.1.

TABLE 6

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 180° F., homogenized, and sterilized

Storage *(wk.)*	Casein component	Temperature					
		40° F.		70° F.		100° F.	
		D[b]	A[c]	D[b]	A[c]	D[b]	A[c]
		Mobility *(cm.^{2} sec.$^{-1}$ v^{-1} × 10^{-5})*					
Raw	α			−6.00	−6.86		
	β			−3.14	−4.28		
	γ				−3.09		
0	α^a			−5.84	−6.80		
	β			−3.23	−4.31		
4	α^a	−6.34	−6.81	−6.43	−6.95	−6.49	−7.01
	β	−3.46	−4.21	−3.56	−4.25	−3.39	−4.31
8	α^a	−5.85	−6.73	−6.38	−7.25	−5.91	−6.67
	β	−3.22	−4.10	−3.34	−4.56	−3.39	−4.15
	X						−2.92
12	α^a	−6.20	−6.89	−6.20	−6.89	−6.26	−7.02
	β	−3.38	−4.30	−3.44	−4.25	−3.53	−4.33
	X						−2.84
20	α^a	−6.04	−6.88	−5.90	−6.74	−6.04	−6.77
	β	−3.35	−4.19	−3.26	−4.16	−3.35	−4.08
	X		−2.80		−2.75		−2.52
		Relative area *(%)*					
Raw	α			73.9	68.7		
	β			26.1	29.6		
	γ				1.7		
0	α^a			80.1	72.1		
	β			19.1	27.9		
4	α^a	79.4	75.2	80.7	76.8	81.8	77.0
	β	20.6	24.8	19.3	23.2	18.2	23.0
8	α^a	79.3	75.8	81.9	77.8	81.6	78.2
	β	20.7	24.2	18.1	22.2	18.4	20.5
	X						1.3
12	α^a	83.2	81.7	81.6	78.3	83.8	77.5
	β	16.8	18.3	18.4	21.7	16.2	19.7
	X						2.8
20	α^a	83.2	77.0	81.9	78.2	85.0	76.8
	β	16.8	20.4	18.1	18.5	15.0	18.7
	X		2.6		3.3		4.5

[a] α-Casein plus denatured serum proteins.
[b] Descending.
[c] Ascending.

TABLE 7

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 140° F., sterilized, and homogenized

Storage (*wk.*)	Casein component	Temperature					
		40° F.		70° F.		100° F.	
		D[b]	A[c]	D[b]	A[c]	D[b]	A[c]
		Mobility (*cm.² sec.⁻¹ v⁻¹ × 10⁻⁵*)					
Raw	α			−6.00	−6.86		
	β			−3.14	−4.28		
	γ				−3.09		
0	α			−5.95	−6.80		
	β			−3.23	−4.30		
4	α^1		−6.87	−6.29	−6.87		
	α^a	−6.41				−6.28	−6.93
	α^2		−7.34	−6.87	−7.16		
	β	−3.55	−4.43	−3.55	−4.43	−3.49	−4.37
	X		−2.97		−2.91	−2.15	−2.50
8	α^1		−6.44		−6.32		
	α^a	−6.03		−6.44		−6.03	−6.61
	α^2		−6.84		−6.78		
	β	−3.48	−4.12	−3.65	−4.17	−3.48	−4.17
	X		−2.96		−2.84	−2.15	−2.78
12	α^1		−6.43	−5.51	−6.67		−6.41
	α^a	−6.03				−6.00	
	α^2		−6.89	−6.15	−7.19		−6.92
	β	−3.39	−4.25	−3.25	−4.29	−3.48	−4.27
	X				−2.48		−2.89
20	α^a	−6.09	−6.89	−6.09	−6.72	−5.97	−6.78
	β	−3.56	−4.31	−3.50	−4.25	−3.39	−4.19
	X	−2.13	−3.04	−1.95	−2.76	−1.95	−2.76
		Relative area (*%*)					
Raw	α			73.9	68.7		
	β			26.1	29.6		
	γ				1.7		
0	α^a			79.4	73.0		
	β			20.6	27.0		
4	α^1		32.5	41.9	37.0		
	α^a	79.1				83.0	76.6
	α^2		40.5	42.1	37.4		
	β	20.9	25.0	16.0	25.2	15.2	20.2
	X		2.0		0.4	7.8	3.2
8	α^1		39.5		44.3		
	α^a	79.8		86.4		85.0	79.1
	α^2		33.4		33.9		
	β	20.2	24.3	13.6	18.9	12.7	15.4
	X		2.8		2.9	2.3	5.5
20	α^1		38.8	27.1	47.2		38.7
	α^a	79.3				91.6	
	α^2		38.0	58.3	36.1		42.9
	β	20.7	23.2	14.6	15.3	8.4	12.1
	X				1.4		6.3

[a] α-Casein plus denatured serum proteins.
[b] Descending.
[c] Ascending.

Sterilization decreased the mobility of α-casein in a buffer of pH 8.7 and an ionic strength of 0.1, either by changing its zeta potential or by causing it to associate with the denatured serum proteins (Tables 2, 3, 5, 6, 7, and 8). Tobias *et al. (19)* observed a decrease in the mobility of α-casein in a buffer of pH 8.7

and an ionic strength of 0.1. Variations in mobilities of the casein from different raw milks were observed and this may be due to the relative amounts of α_1 and α_2 casein *(2)*.

Sterilization probably did not affect either the mobility (Tables 2, 3, 5, 6, 7, and 8) or the real concentration of β-casein (Table 4), except in Table 3, where

TABLE 8

Effect of time and temperature of storage on the electrophoretic properties of casein from milk preheated to 180° F., sterilized, and homogenized

Storage *(wk.)*	Casein component	Temperature: 40° F. D[b]	40° F. A[c]	70° F. D[b]	70° F. A[c]	100° F. D[b]	100° F. A[c]
		Mobilities *(cm.^{2} sec.$^{-1}$ v^{-1} × 10^{-5})*					
Raw	α			−6.00	−6.86		
	β			−3.14	−4.28		
	γ				−3.09		
0	α^a			−5.89	−6.57		
	β			−3.46	−4.08		
4	α^a	−6.38	−6.84	−6.26	−6.72	−6.49	−7.01
	β	−3.48	−4.23	−3.59	−4.17	−3.59	−4.23
8	α^a α^1				−6.67		
	α^a	−5.85	−7.08	−6.08		−5.85	−6.96
	α^a α^2				−7.20		
	β	−3.28	−4.39	−3.39	−4.27	−3.28	−4.27
	X						−2.87
12	α^a α^1			−5.84	−6.41		
	α^a	−6.01	−6.74			−6.09	−7.01
	α^a α^2			−6.35	−6.86		
	β	−3.37	−4.16	−3.40	−4.19	−3.27	−4.31
20	α^a α^1						−6.35
	α^a	5.72	−6.74	−6.06	−6.74	−6.01	
	α^a α^2						−6.96
	β	−3.15	−4.27	−3.36	−4.16	−3.32	−4.17
	X		−2.75		−2.53		−2.64
		Relative area *(%)*					
Raw	α			73.9	68.7		
	β			26.1	29.6		
	γ				1.7		
0	α^a			78.5	73.7		
	β			21.5	26.3		
4	α^a	80.5	75.4	82.4	76.4	80.8	75.7
	β	19.5	24.6	17.6	23.6	19.2	24.3
8	α^a α^1				40.3		
	α^a	81.7	73.8	79.9		82.7	72.7
	α^a α^2				34.5		
	β	18.3	26.2	20.1	25.2	17.3	22.7
12	α^a α^1			31.9	29.3		4.6
	α^a	81.7	76.5			82.9	
	α^a α^2			50.5	51.7		80.4
	β	18.3	23.5	17.6	19.0	17.1	19.6
20	α^a α^1						43.7
	α^a	81.3	77.8	84.4	75.2	83.7	
	α^a α^2						36.4
	β	18.7	20.5	15.6	21.5	16.3	17.2
	X				3.3		2.7

[a] α-Casein plus denatured serum proteins.
[b] Descending.
[c] Ascending.

a decrease in the mobility of β-casein was observed. This agrees with the results reported by Tobias *et al. (19)* for skimmilk sterilized at 300° F. and at a pH of 8.7 and an ionic strength of 0.1, but Slatter and Van Winkle *(17)* reported a decrease in the mobility of β-casein.

Sterilization did not cause hydrolysis of the proteins to nonprotein nitrogen, because there were no changes in the nonprotein nitrogen values of raw or sterilized milk. Menefee *et al. (11)* reported that some hydrolysis occurred when evaporated milk was processed, but pasteurization at 145° F. for 30 min. produced no changes in the nitrogen distribution *(21)*.

During storage, the amount of α-casein plus denatured serum proteins did not change greatly (Table 4). The amount of β-casein in the casein from stored milks varied with the preheating temperature, homogenization, and temperature of storage.

Statistical analyses of the results showed that the relative areas of α-casein and β-casein components in casein from different milks, and for the same milks stored at different temperatures, were significantly different from the relative

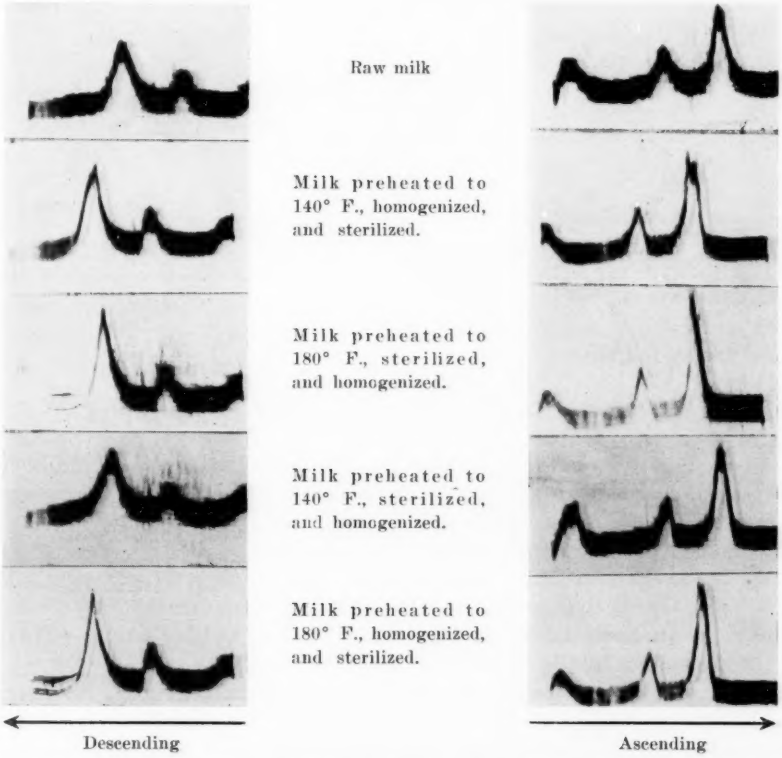
Raw milk
Milk preheated to 140° F., homogenized, and sterilized.
Milk preheated to 180° F., sterilized, and homogenized.
Milk preheated to 140° F., sterilized, and homogenized.
Milk preheated to 180° F., homogenized, and sterilized.
Descending
Ascending

FIG. 1. Electrophoretic patterns of casein from raw milk and freshly sterilized milk.

areas of the unstored milks, which indicated changes in the composition of casein in stored milks. Since no nitrogen determinations were made on the samples of milks (Tables 5 to 8), it was assumed that the changes observed in the nitrogen distribution in these caseins would be similar to those observed in Table 4.

The breakdown of β-casein was greatest in casein from milk preheated to 140° F. and stored at 100° F., less at 70° F., and least at 40° F. (Tables 2, 4, and 5). The breakdown of β-casein was less when milk was homogenized after sterilization (compare Tables 2 and 5 with Table 7).

When milk was preheated to 180° F., the breakdown of β-casein was less; while it occurred in the casein from milk stored at 100° F., the results were uncertain at lower temperatures. However, the β-casein nitrogen was lower at the end of the storage period (Table 4).

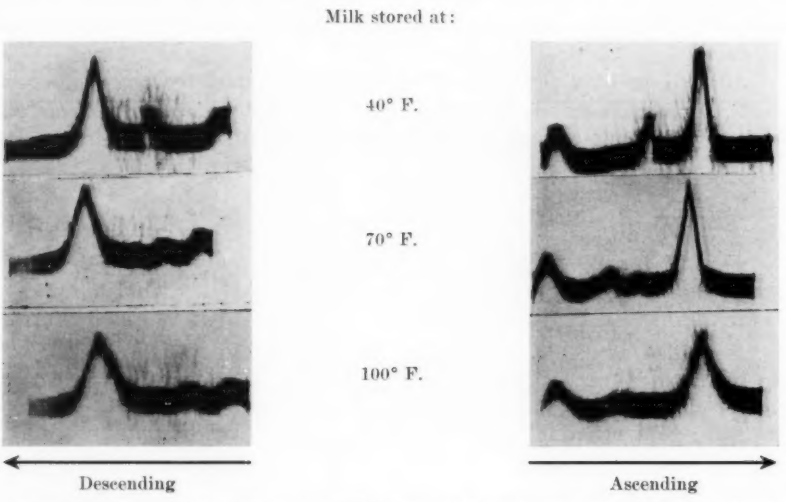

FIG. 2. Electrophoretic patterns of casein from milk preheated to 140° F., homogenized, sterilized, and stored for 20 wk.

The breakdown of β-casein in casein from stored milks resulted in a new component designated as X, which appeared in the electrophoretic patterns. It is evident from Tables 2, 5, and 7 that the X-component appeared at all temperatures of storage when milk was preheated to 140° F., homogenized either before or after sterilization. The X-component appeared more frequently on the ascending than on the descending side of the patterns, especially at the lower storage temperature. Its occurrence at 40° F. storage, either on the ascending or the descending side, was not consistent.

When milk was preheated to 180° F., homogenized, sterilized, and stored, the X-component in casein was not as large as at 140° F., appearing to a greater extent at 100° F. than at 70° F., and few times at 40° F. (Tables 3, 6, and 8). Again, it appeared more often on the ascending than on the descending side.

This X-component, whenever it occurred on the descending side, moved with a lower mobility than that of β-casein and at approximately that of γ-casein. On mobility alone it can not be identified as γ-casein without establishing its physical and chemical properties *(6)*. It would be interesting to isolate the X-component for further study.

Further, during storage a greater increase in the noncasein nitrogen was found in milk preheated to 140° F., homogenized, and sterilized (Table 1). This increase in noncasein nitrogen was greatest at 100° F. storage, intermediate at 70° F., and much less at 40° F. Again, this could be accounted for by the breakdown of β-casein (Table 4) to smaller protein units which were not precipitated as casein and contributed to increased values for noncasein nitrogen in the filtrate.

Storage at 100° F. of milk preheated to 140° F., homogenized, and sterilized resulted in an increase in the nonprotein nitrogen (Table 1). It is postulated that at 100° F. storage the β-casein was broken down to an entity which was filterable as nonprotein as well as the X-component and noncasein nitrogen. At lower temperatures of storage, there was no change in the nonprotein nitrogen, even though at 70° F. storage, β-casein was still breaking down (Table 4). Apparently, the X-component and the noncasein nitrogen components are the only products of this breakdown. Shahani and Sommer *(15)* reported an increase in nonprotein nitrogen in evaporated milk stored at 100° F.

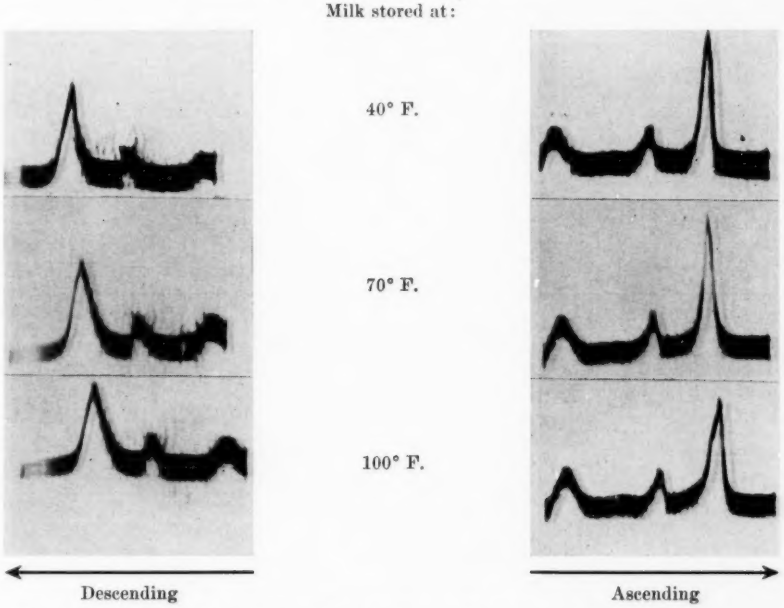

FIG. 3. Electrophoretic patterns of casein from milk preheated to 180° F., homogenized, sterilized, and stored for 20 wk.

The breakdown of β-casein may be due to dissociation, chemical hydrolysis, or enzyme hydrolysis. Dissociation seems unlikely because, to produce nonprotein materials, the basic association unit would have to be nonprotein in nature. Chemical hydrolysis is unlikely, because the stored milk had a reaction so near neutrality. Moreover, it is unusual that chemical hydrolysis would be selective for different components of casein.

It is proposed that a protease specific for β-casein either may have survived the heat treatment or may have been reactivated during storage. Thus, the breakdown of β-casein to nonprotein materials could be more logically explained. The extent to which this occurred depended upon the preheating temperature, homogenization, storage temperature, and time. From Tables 1, 2, 4, 5, and 7, it is seen that when milk was preheated to 140° F., homogenized, sterilized, and stored, the following changes were observed: a greater decrease in the concentration of β-casein and an increase in the noncasein and nonprotein when compared with milk preheated to 180° F. Of the three temperatures, 100° F. seems to be

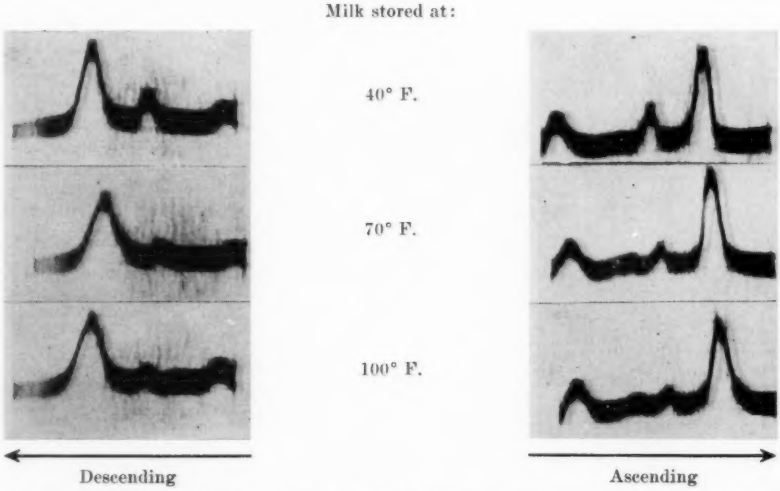

FIG. 4. Electrophoretic patterns of casein from milk preheated to 140° F., sterilized, homogenized, and stored for 20 wk.

best for the activity of the enzyme. Preheating milk at 180° F. slightly changed the β-casein or nonprotein nitrogen. It is conceivable that preheating at 140° F. and sterilizing at 305° F. reversibly or incompletely denatures the enzyme, enabling it to hydrolyze the β-casein, but that preheating at 180° F. either more completely inactivates or irreversibly denatures the enzyme.

Hipp *et al. (6)* found that the protease in milk is concentrated in the α-casein fraction. Warner and Polis *(23)* showed that this enzyme is resistant to heat, though it can be destroyed by heating in a borax solution at 80° C. for 10 min. They suggest that a concentrated protease be prepared and that its activity,

selectivity, optimum pH, and temperature be investigated with pure fractions of casein to characterize the enzymatic process. It has been shown that the enzyme, phosphatase, is reactivated in milk *(3, 24)*.

It can be readily seen (Figure 1) that there was a tendency for a split to occur in α-casein peak. Tobias *et al. (19)* observed in the rising pattern of skim-milk that α-casein split into three components at pH 8.7 and ionic strength of 0.1. Slatter and Van Winkle *(17)* observed that α-casein migrated as two distinct components, α_1 and α_2, over a pH range above 4.2 and ionic strengths of 0.02 and 0.1. In the casein from milk preheated to 140° F., homogenized, and sterilized, two peaks were observed in α-casein which were more pronounced on the ascending side (Figure 1) and were reported as α_1 and α_2. The split also appeared in casein from the milk preheated to 140° F., homogenized, sterilized, and stored at all temperatures (Table 5), but there was no consistency in its occurrence even in the same milk or at the same storage temperature. During storage, the casein from milk preheated to 180° F., homogenized, and sterilized showed no tendency to split (Tables 3 and 6). This split phenomenon was not studied, but it may be due to differences in milks and processing conditions. Warner *(22)* sometimes observed a split on the ascending side of α-casein when the protein concentration was less than 1%.

The electrophoretic mobility data were not statistically analyzed. During storage, only occasional variations were observed in the mobilities of α-casein

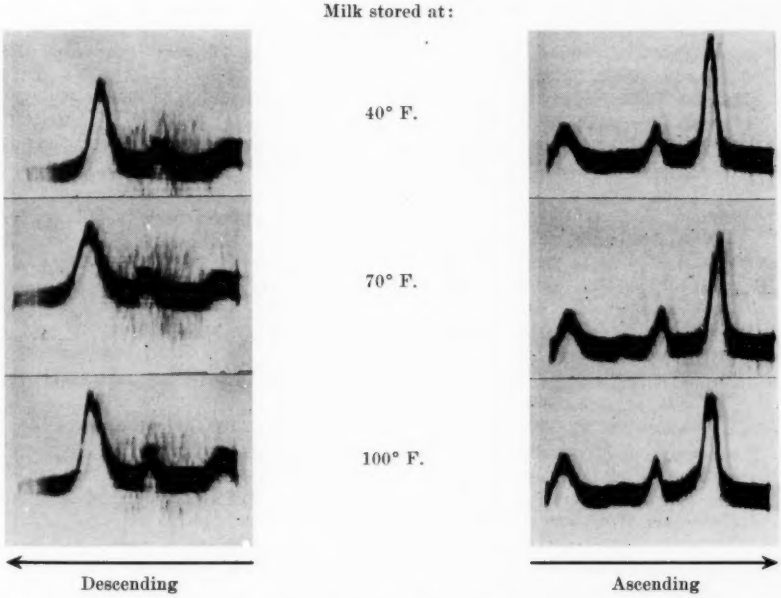

FIG. 5. Electrophoretic patterns of casein from milk preheated to 180° F., sterilized, homogenized, and stored for 20 wk.

plus denatured serum proteins and β-casein in casein from milk preheated to 140° F., homogenized before or after sterilization. The mobilities remained the same or were only slightly altered (Tables 2, 5, and 7) at the end of the storage period.

In casein from milk preheated to 180° F., homogenized either before or after sterilization during storage, wide variations were observed in the mobilities of α-casein plus denatured serum proteins. These variations in mobility were greater in the α-casein component plus denatured serum yielding two peaks.

During storage, the milks which were preheated to 140° F., homogenized either before or after sterilization, and the milks preheated to 180° F., sterilized, and homogenized showed no cream layer or sediment. However, a thick protein-fat sediment was observed in milk preheated to 180° F., homogenized, sterilized, and stored at 40, 70, and 100° F. This milk showed four to five distinct horizontal layers in different parts of the glass container at the end of storage at all temperatures and was difficult to redisperse. However, no correlated changes could be observed in the electrophoretic patterns of casein from milk preheated to 180° F., which could explain these differences that appear to be associated with the order of processing. Homogenization after sterilization probably stabilized the proteins in milk preheated to 180° F. These observations were not confirmed with quantitative data. Such studies are in progress.

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STUDIES ON THE RELATIVE BACTERIAL CLEANABILITY OF MILK-CONTACT SURFACES

E. B. MASUROVSKY AND W. K. JORDAN
Department of Dairy Industry, Cornell University, Ithaca, New York

SUMMARY

These studies are concerned with the removal of bacteria, deposited with various soils, on milk-contact surfaces. The bacteria were labeled with Phosphorus^{32}, incorporated into test soils, dried down with the soils upon various milk-contact surfaces under highly reproducible conditions, removed by a standard cleaning procedure, and assayed by the agar-submersion, Geiger-counting, and autoradiographic techniques. The data so derived were tested for significance by an Analysis of Variance, and grouped by the Duncan Multiple Range Test. The results show statistically significant differences in cleanability, between different surface finishes applied to a given material and between different materials having comparable finishes. Organisms suspended in a homogenized whole milk soil were much more easily removed from the surfaces studied than organisms suspended in an 0.85% saline solution. Multiple soiling and washing of a surface results in a general increase in the number of bacteria retained by a surface. Brushing was found to be effective in cleaning smooth polished, ground, and molded surfaces, and relatively ineffective in removing bacteria entrapped in the surface features of cold rolled, abraded, blasted, and porous surfaces.

On the basis of all the tests performed, the surface finishes which displayed the greatest ease of cleanability were the highly polished, nonporous surfaces. The finely ground and smoothly molded finishes followed close behind, and the cold rolled, abraded, blasted, and porous finishes were the most difficult to clean. Under the conditions of testing utilized in these experiments, the direct agar-submersion technique proved to be ineffective as a means of accurately determining the relative cleanability of different surface finishes and of different materials. The radiological counting procedure proved to have higher reproducibility than agar-submersion methods for estimating the numbers of bacteria remaining on a cleaned surface.

For many years, a difference of opinion has existed regarding the relative cleanability of different materials, and the surface finishes applied to materials, which contact dairy products during their processing and merchandising. The traditional method of studying this in the Dairy Industry has been to ascertain the numbers of viable bacteria present on milk-contact surfaces, and to use such data as indices of how well the surfaces were cleaned. The works of Hammer and Olson *(5)*, Fellers, Levine, and Harvey *(4)*, Walter and Hucker *(12)*, Moore *et al. (9)*, and Hansen and Winder *(6)* have contributed greatly to an understanding of potentialities and limitations of techniques based upon such an approach. The results of these investigations clearly demonstrated that while useful as field tests, the techniques employed were subject to analytical errors when applied to a quantitative evaluation of residual bacteria on different kinds of materials possessing widely diverse surface finishes.

In other fields, the problem has been approached from the point of view of the soil-retaining capacity of a surface. Numerous procedures have been developed to measure the amount of soil remaining on a surface after cleaning. Photometric techniques [Mallman *et al. (8)*] have been used extensively in this

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work, as have fluorescent dyes [Domingo *(2)*] and various "water-break" tests. Generally, these techniques have been found to be lacking in sensitivity and reliability at low levels of soil residues.

Radioisotopic techniques now exist which avoid the pitfalls of the former techniques and possess high sensitivity at both high and low soil residue levels. Moreover, these techniques make possible the testing of a wide range of materials and surface finishes in a highly reproducible and directly comparable manner. Not only can the quantity of soil be accurately determined by these techniques, but the pattern of soil distribution on a surface can be clearly shown. Ridenour and Armbruster *(10)* demonstrated the utility of radiological techniques for the determination of relative concentrations and location of $Phosphorus^{32}$-labeled bacteria on a variety of materials in their studies on the cleanability of eating utensils. Based upon this and other previous work, a rapid, sensitive, and highly reproducible method was developed for determining the relative cleanability of different materials, and surface finishes applied to materials, that is used in the fabrication of dairy equipment and dairy product containers. This paper reports on the investigations carried out using this method for the determination of the removal of soils bearing $Phosphorus^{32}$-labeled *Escherichia coli* and *Micrococcus pyogenes* var. *aureus* from a variety of milk-contact surfaces.

EXPERIMENTAL PROCEDURE

Materials studied and preparation of materials for testing. Major emphasis was placed on the "300 series" of stainless steels. One dozen commercial finishes and one laboratory-produced finish were studied. Among the other materials tested were nickel-base alloys, copper-base alloys, aluminum alloys, glasses, and plastics. A description of the type and surface finishes of these materials is presented in the tables of experimental results. The specimens tested were flat, 1-in. diameter discs which were either cut from sheets of the materials on a lathe, ground out of sections of glass with a carborundum-turpentine abrasive mixture, or supplied as such by the fabricators of the materials. This specimen size and configuration were found to be advantageous for radiological assaying, soiling and cleaning, and submerging in agar for bacterial colony determinations.

Prior to testing the samples of stainless steel, nickel-base alloys and copper-base alloys were given three different soak-clean treatments, a solvent degreasing, a vapor degreasing, a reverse electro-cleaning, an acid dip, and a thorough rinsing in distilled water.

The aluminum alloys were given comparable cleaning treatments prior to conditioning the surfaces for the controlled reformation of the natural oxide film.

The glass samples were degreased, scrubbed with a precipitated calcium carbonate slurry, dipped in "Aqua Regia," and rinsed in distilled water.

After cleaning with an appropriate mixture of detergents, plastic samples were dried at 60° C. for 2 hr. Glass and metal samples were dried at 80° C. for 1 hr.

All soak-cleaning operations were carried out with constant agitation. Speci-

mens were individually wrapped in clean lens tissue after cooling to room temperature, and stored in desiccators until used.

Surface analysis. A Brush R.M.S. meter, Model BL106, was used in conjunction with surface analyzer, Model BL103, to determine the R.M.S. value of the surface of all samples. This is the root mean square of an infinite series of distances measured in microinches (μ'') from the hypothetical nominal or mean surface line to the actual irregular surface contour.

Microorganisms used and preparation of test soils. Two organisms were used in this series of experiments: *Escherichia coli* A.T.C. #11229 and *Micrococcus pyogenes* var. *aureus* A.T.C.C. #6538. Radioactive cultures of these bacteria were prepared by transferring a loop inoculum from a fourth consecutive daily transfer of the cells, grown in nutrient broth and peptone broth *(1)*, respectively, into 20 ml. of either broth contained in silicone-treated 250-ml. Erlenmeyer flasks.

The high specific activity form of Phosphorus^{32} was added to the media to give a concentration of 10 μc. Phosphorus^{32} per ml. The inoculated flasks were incubated with constant agitation at 30° C. for 14 hr. for *Escherichia coli* and 18 hr. for *Micrococcus pyogenes* var. *aureus*. These cultures were aseptically transferred to sterile, silicone-treated 17-mm. Pyrex centrifuge tubes and centrifuged for 5 min. at 12,000 r.p.m. Following the aseptic decanting of radioactive media, the organisms were treated to remove adsorbed Phosphorus^{32}. This was done by washing the organisms in 10-ml. portions of sterile 0.85% saline until the activity of the filtered supernatant was reduced to less than 10 counts/min/25 ml. These organisms were then resuspended in sterile 0.85% saline and kept under refrigeration at 4° C. until used.

The Phosphorus^{32}-labeled bacteria were suspended in two kinds of soils: pasteurized homogenized whole milk (3.6% B.F., 8.8% S.N.F.) and sterile 0.85% saline solution. The organisms were incorporated into the soils by transferring the liquid soil carrier to a centrifuge tube containing a washed bacterial pellet and breaking up the pellet in the soil carrier with sterile glass beads. These bacterial soil suspensions were then transferred to a sterile siliconed-glass mixing-chamber and stirred with a magnetic stirrer to give uniform suspensions. Concentrations of about 2½ billion cells per milliliter of soil were used.

Apparatus used in soiling and cleaning specimens. There were two special pieces of equipment used in this study: a device for soiling the surfaces of the specimens in a reproducible manner, and a mechanical washing device for cleaning the soiled specimens under standard conditions. Soiling of a sample disc was accomplished by pipetting 0.05 ml. of bacterial soil suspension onto the center of the disc, which was mounted on the turntable of the soiling device, and mechanically spreading the soil over the surface of the disc by means of a siliconed-glass rod which traversed the disc as it rotated. The soil film was dried in two stages: first, for 6 min. at 60° C. by means of an infrared lamp and second, for 10 min. at 80° C. in an oven. Soil coatings produced in this way were not necessarily perfectly uniform in thickness over the surface of the disc, but were highly reproducible, which was more important for the over-all accuracy

of the determinations. It has been found that sets of 12 discs of the same material selected at random in a soiling operation gave maximum variations of ± 3% in initial counts of radioactivity. It migh be expected that the sum of the counting error and the uncertainty in measurement of this small volume of soil would account for that amount of variation. Autoradiograms made of the soiled discs showed the distribution and concentration of the tagged bacteria to be relatively uniform over the surfaces of the discs. These specimens were aged for 16 hr. at 25° C. prior to cleaning. Previous experimentation indicated that aging increased the soil-substrate bond materially over that of a freshly soiled surface.

The soiled discs were cleaned in a turntable-mounted glass tank. A disc was positioned in the center of a stainless steel plate in the bottom of the tank and submerged in 400 ml. of alkaline cleaning solution[1] (0.03% active alkalinity) maintained at 120° F. As the disc rotated, it was scrubbed by a special nylon brush or a fine-grained photographic sponge (duPont #4A) mounted on a weighted cleaning arm (167 g.) which traversed the disc with a windshield-wiper motion. A cleaning time of 1⅛ min., equivalent to 36 passes of the cleaning arm, was used. Following cleaning, the discs were rinsed with 400 ml. of distilled water, dried in an 80° C. oven, cooled, and stored in desiccators until assayed.

Radioassaying procedures. The concentration and distribution of labeled bacteria were determined after soiling and, following the cleaning and drying procedures, by counting the beta particles emitted by the disintegrating Phosphorus^{32} with an end-window Geiger-counting assembly (Tracerlab Model TGC 3A argon-filled 1.4 mg/cm^2 end-window Geiger tube connected to a Nuclear Model 162 Scaler) and by X-ray autoradiography, using Ansco "Super Ray B" X-ray film, Emulsion No. 26C273-8-273. Counting was done at constant geometry (same specimen surface-to-tube distance and position) for various periods of time, depending upon the activity level (counting error ± 2% $\bar{x}$; p = 0.10). Autoradiogram exposure times of 48 hr. for soiled specimen discs and 168 hr. for cleaned discs were used in all of the experiments reported.

Bacteriological procedures. In addition to the radiological methods used for the determination of bacterial residues, representative test discs, which were given treatments identical to those used on the radiological specimens, were aseptically transferred immediately after washing to sterile Petri dishes and submerged in agar. Levine E.M.B. Agar was used for *Escherichia coli* and Chapman's Staphylococcus Agar No. 110 was used for *Micrococcus pyogenes* var. *aureus*. The purpose of these determinations was to compare the radiological procedures with a sensitive conventional method. By observing and enumerating

[1] Cleaner compositions

	(%)
Cleaner for all specimens except aluminum:	
Soda Ash	55
Sodium Metasilicate	35
Sodium Tripolyphosphate	8
Sodium Alkyl Aryl Sulfonate	2
Cleaner used on aluminum:	
Sodium Bicarbonate	30
Sodium Metasilicate	60
Sodium Tripolyphosphate	8
Sodium Alkyl Aryl Sulfonate	2

the colonies which developed on the surface of the test discs after an incubation period of 48 hr. at 37° C., it was hoped that some correlation could be made between the relative concentration and distribution of the organisms shown by colony growth and by the radiological techniques.

Experimental design and analysis of data. The experimental design used in this series of tests was highly randomized in nature. The order in which the test discs were soiled and washed within a given experiment was determined by the use of a table of random numbers. A minimum of four discs of each type of specimen was run in a given experiment, except in the case of the abraded specimens, when only two discs of each were used. Radioactive counts on three out of the four discs were used in the statistical analyses. The fourth disc was used in the agar submersion determinations. The abraded specimens were not analyzed statistically because they were intended to be used only for the purposes of general comparison.

Statistical analyses were performed on data derived from the primary radioactive disintegration counting rates (counts/minute), which were corrected for coincidence, decay, and back-scatter to the equivalent of optically polished Corning #7740 Pyrex glass. The corrected data were transformed to values representing the logarithm to the Base 10 of the counts, plus 0.1. A value of 0.1 was added to all the data, to allow for the inclusion of counts of 1 above background in the statistical analyses.

These transformed data were first subjected to an analysis of variance, as described by Snedecor *(11)*, on an experiment-wide basis to ascertain whether a significant difference existed between the cleanability of all of the specimens. While the calculated variance ratios (F values) [see Table 1], determined at the 5% testing level, indicated that significant differences did exist between various specimens, they did not show where the differences were.

The Multiple Range Test of Duncan *(3)* was selected to provide that information beween finishes applied to a particular type of material for a given experiment and treatment. The application of this analysis to the data on a within-materials basis was intended to bring out the important differences in cleanability contributed by the surface finishes of the materials and, at the same time, to present the data in as clear and objective a manner as the experimental design permitted. However, the data, as presented in the tables, are directly comparable between materials as well as between finishes and, furthermore, are adjusted so that interexperimenal analysis is possible. A further description of the treatments given the data, together with directions for the interpretation of the statistical groupings, is provided at the bottom of the tables of experimental results.

EXPERIMENTS PERFORMED

The five experiments in this series of tests[2] were as follows:

[2] Soil removed from substrates in Experiments I–IV by washing with a duPont Fine-Grained Cellulose Sponge (4-A), using alkaline dairy cleaners previously described.

TABLE 1

Analyses of variance computations for the experiments performed

Source of variance	Degrees of freedom	Sum of squares ($Log_{10}[x + 0.1]$)	Mean square ($Log_{10}[x + 0.1]$)	Calculated variance ratio (F value)
Experiment I				
Between materials	35	11.9405	0.3412	5.4[a]
Within materials	72	4.5854	0.0637	
Totals	107	16.5259		
Experiment II				
Between materials	54	62.0169	1.1485	35.9[a]
Within materials	110	3.5175	0.0320	
Totals	164	65.5344		
Experiment III				
Between materials	54	59.7552	1.1066	263.5[a]
Within materials	110	0.4666	0.0043	
Totals	164	60.2218		
Experiment IV				
Between materials	54	57.7518	1.0695	354.3[a]
Within materials	110	0.3314	0.0030	
Totals	164	58.0832		
Experiment V				
Between materials	36	5.5200	0.1533	129.4[a]
Within materials	74	0.0878	0.0012	
Totals	110	5.6078		

[a] The probability of obtaining an F value of this magnitude is less than 5%. From this large F value it can be assumed that differences do exist between materials.

Experiment I. A single soiling of test substrates with an *Escherichia coli* suspension in homogenized whole milk.

Experiment II. A single soiling of test substrates with a *Micrococcus pyogenes* var. *aureus* suspension in homogenized whole milk.

Experiment III. A single soiling of test substrates with an 0.85% saline suspension of *Micrococcus pyogenes* var. *aureus.*

Experiment IV. A double soiling of test substrates with an 0.85% saline suspension of *Micrococcus pyogenes* var. *aureus.*

Experiment V. Cleaning of test substrates soiled with an 0.85% saline suspension of *Micrococcus pyogenes* var. *aureus* with a nylon brush.

RESULTS

Dairy metals. Stainless steel: As can be seen (Table 2), when homogenized whole milk was used as the bacterial suspension vehicle there was a much lower level of soil retention than when 0.85% saline was the bacterial carrier. In the experiments utilizing homogenized whole milk, there was little difference to be found between the finishes, the major divisions occurring between ground and polished finishes, cold-rolled sheet finishes, and vapor and sand-blasted surfaces. However, in the experiments in which 0.85% saline was the soil carrier, there resulted a much finer breakdown between finishes, as well as greater soil retention by the discs.

The data indicate that specimens possessing a No. 4 polish, or better, were in the top cleanability groupings in all five experiments. These groupings neces-

TABLE 2

Relative bacterial cleanability of different surface finishes applied to stainless steel alloys

Type of material and surface finish	Surface analyzer reading (μ" R.M.S.)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V	
		Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]
Types 302 and 304											
No. 8 (Mirror)	0.5–1.2	3	*1*	3	*1*	94	*1*	185	*1*	71	*1*
No. 7 (High luster polish)	1.0–5.0	3	*1*	3	*1*	99	*1*	190	*1*	71	*1*
Electro polish	7.0–9.0	3	*1*	4	*1*	119	*1, 2*	192	*1*	72	*1*
240-grit finish	17–20	3	*1*	4	*1*	123	*1, 2*	200	*1*	93	*1, 2*
No. 6 (Tampico brushed)	5.0–8.0	3	*1*	4	*1*	160	*1, 2, 3*	233	*1*	119	*1, 2, 3*
No. 4 (Standard "commercial")	8–12	4	*1*	4	*1*	165	*1, 2, 3*	247	*1*	124	*1, 2, 3*
No. 3 (Rough polish)	9–21	4	*1*	5	*1*	203	*2, 3*	262	*1*	164	*2, 3*
Mill finish	8–13	4	*1*	10	*2*	265	*3*	757	*2*	200	*3*
2B (Bright cold rolled)	8–21	8	*2*	15	*2, 3*	670	*4*	897	*2*	611	*4*
2D (Dull cold rolled)	5–32	11	*2*	19	*3*	798	*4*	1,121	*2, 3*	650	*4*
Vapor blast	42	12	*2, 3*	19	*3*	884	*4*	1,316	*2, 3*	816	*4*
Sand blast	140	21	*3*	26	*3*	1,173	*4*	1,827	*3*	1,115	

[a] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

[b] Computations performed at the 5% testing level, using the over-all Error Mean Square value for the determination of the Standard Error of a Treatment Mean. Specimens having the same group number are not significantly different from each other, but are significantly different from those in another group, provided that they are not contained in a subset (indicated by two or more group numbers) of the groups being compared.

sarily changed from one experiment to another, and in some there is an overlapping of the groupings. Thus, while the No. 4 finish is in the first grouping in Experiments I, II, and IV, it may be considered as being in Group 1, 2, or 3 in Experiments III and V. There is, therefore, a statistical difference between the cleanability of the No. 4 finish and the higher polishes. However, on a practical basis, this difference is probably not too important, since the No. 4 finish also groups with the higher polish finishes.

The count of radioactivity due to the bacteria remaining after cleaning was used in the statistical analyses rather than the percentage removal of organisms during the cleaning. If per cent removal had been used as the index of performance of a surface, distinctions would not be nearly as evident since, even in the experiments in which the soils were the most difficult to remove, better than 90% removal from all surfaces was effected.

The statistical analysis was also useful in throwing light on the controversial question of correlation of surface profile values and cleanability.

Noteworthy among the results obained along these lines were the performances of the Electro Polished and 240-grit finishes which exhibited better cleanability than some of the ground and polished finishes with lower R.M.S. values. The lack of a close correlation between R.M.S. values and cleanability is clearly brought out by these results and others presented in succeeding tables.

Abrasion of a No. 7 finish applied to Type 302 stainless steel greatly reduced its cleanability characteristics (See Table 7).

Nickel-base alloys: The reactions of the Inconel and Monel specimens evaluated in this study are interesting, because they further refute the criterion of R.M.S. value as a guide to cleanability, and strongly suggest the importance of the physical and chemical make-up of a material upon its soil-retaining capacity. Although in the majority of cases there was found to be no statistically significant differences between the Inconel and Monel specimens by the Duncan Multiple Range Test (see Table 3), the values obtained indicate a greater soil retention by the smoother Monel than by a rougher Inconel counterpart. Even when abraded (see Table 7), the same difference between materials was evident. However, more work should be done on this category of materials, because of the limited number of samples that were available for testing.

Copper-base alloys. The specimens consisted mainly of casting alloys used in the fabrication of such dairy plant equipment as pipe fittings, valves, and sanitary pump elements. Tables 2 and 3 show that these materials exhibited a cleanability similar to stainless steel on a comparable finish basis, when free from tarnish and other corrosion products. However, in the corroded state (produced by a 5% w/v HNO_3 dip and water soak, followed by air drying), the soil-retention capacity of the specimens increased materially. This increase is especially significant, because it further stresses the importance of the proper cleaning procedures to minimize chemical attack upon these materials. Abrasion of the specimens resulted in a somewhat higher soil retention than that of the unmarred condition.

TABLE 3

Relative bacterial cleanability of nickel- and copper-base dairy alloys

Type of material and surface finish	Surface analyzer reading (μ" R.M.S.)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V	
		Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radioactivity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]
Nickel-base alloys:											
Inconel	55	7	*1*	8	*1*	275	*1*	328	*1*	243	*1*
Monel	3.0–7.0	7	*1*	31	*2*	378	*1*	435	*1*	276	*1*
Copper-base alloys:											
Casting alloy WKM buff	10	—	—	3	*1*	95	*1*	187	*1*	—	—
Satin	14	—	—	4	*1*	173	*1*	234	*1*	—	—
120 Emery	15	—	—	4	*1*	191	*1*	282	*1*	—	—
Casting alloy LTA No. 4 (standard)	36	—	—	6	*1, 2*	381	*2*	742	*2*	287	*1*
Wrought sheet 18A	13	—	—	7	*2*	544	*2, 3*	827	*2*	431	*1*
Wrought sheet 18A (corroded)	13	—	—	15	*3*	712	*3, 4*	877	*2, 3*	—	—
Casting alloy LTA No. 4 (standard) (corroded)	36	—	—	34	*3*	1,197	*4*	1,487	*3*	—	—

[a] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

[b] Computations performed at the 5% testing level, using the over-all Error Mean Square value for the determination of the Standard Error of a Treatment Mean. Specimens having the same group number are not significantly different from each other, but are significantly different from those in another group, provided that they are not contained in a subset (indicated by two or more group numbers) of the groups being compared.

Aluminum alloys: A variety of wrought sheet alloys, and a casting alloy commonly used in such dairy equipment as butter churns, were studied in this series of tests. There was little difference to be noted between the rolled wrought sheet alloys (see Table 4) over the entire test series, but a definitely significant difference was noted between the rolled and frosted wrought sheet alloys, and the sand-blast finished casting alloy vs. the sheet alloys. Abrasion of these relatively soft alloys resulted in a difficult-to-clean surface, thereby indicating the need for careful handling of equipment made from this type of material.

Dairy glasses. Three types of glasses were studied: soda-lime dairy container glass, Corning #7740 Pyrex glass (for glass pipelines), and an acid-resistant blue glass enamel material used to line such equipment as processing vats. The soda-lime glass specimens were prepared from sections of milk bottles supplied by several manufacturers; the Pyrex specimens were supplied with both an optically polished and irregular molded finish; and the blue glass enamel lining, fused to steel discs, was supplied in both glazed and deglazed surface conditions.

All the glasses studied (see Table 5) were found to be highly cleanable. The optically polished specimen was consistently the most easily cleanable of the series. When these materials were scratched (or deglazed) their soil-retention properties increased markedly, especially in the case of the deglazed blue glass enamel lining material, which possessed a highly porous subsurface (see Table 7). The necessity for careful handling of these materials, to maintain their smoothly fused surfaces, is evident from these data.

Plastics. As can be seen (Table 6), those plastics which possessed a smooth molded finish were generally quite easily cleaned. Certain polyfluorocarbon and polyethylene formulations which were not readily wettable with aqueous solutions were more difficult to clean than their more readily wettable counterparts. This was strikingly illustrated by Polyfluorocarbon Formulation 1 and Polyethylene Formulation 1412-9-A.

Some plastics, which at first glance appeared to have a readily cleanable, smooth surface had, in reality, little pits or pinholes distributed over them. This was true of Polyvinylidene Chloride Formulation 548 and Polystyrene Formulation 5230, and helps account for the greater soil retention of these specimens.

Those specimens which possessed a spongy or porous surface, such as Polyfluorocarbon Laminate SPT and Polyester-Fiberglas Laminate HFL, proved to be difficult to clean. Formulation SPT counted higher than Formulation HFL, due to its greater porosity and low wettability. Another Polyester-Fiberglas Laminate (RHW-1-7), while not possessing a porous surface, had a brittle, jagged surface crisserossed with Fiberglas strands. It retained about three times the amount of soil as Formulation HFL. This could be accounted for by the presence of cavities existing between the Fiberglas strands and the brittle polyester resin. Upon abrading Formulation HFL (see Table 7), a quite porous subsurface was exposed which proved more difficult to clean than a more severely abraded sample of RHW-1-7.

TABLE 4

Relative bacterial cleanability of aluminum alloys used in the dairy industry

Type of material and surface finish[a]	Surface analyzer reading (μ" R.M.S.)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V	
		Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]
Wrought aluminum sheet:											
1,100–0	21–36	—	—	52	*1*	177	*1*	241	*1*	—	—
1,100–H14	24–39	—	—	68	*1*	215	*1*	219	*1*	128	*1*
3,003–H14	28–39	—	—	44	*1*	194	*1*	281	*1*	—	—
5,052–H38	17–32	—	—	68	*1*	192	*1*	238	*1*	—	—
6,053–T6	27–38	—	—	62	*1*	133	*1*	207	*1*	—	—
6,353–T4	30	—	—	46	*1*	121	*1*	157	*1*	113	*1*
6,353–T6	40	—	—	61	*1*	172	*1*	197	*1*	—	—
8 (frosted)	18	266	*1*	506	*2*	737	*2*	781	*2*	683	*2*
Casting alloy:											
43 (Sand blast)	150	1,351	*2*	1,331	*3*	2,208	*3*	3,087	*3*	1,960	*3*

[a] Manufacturer's specification numbers for wrought sheet stock which has been aged and tempered to produce the physical properties characteristic of these alloy designations.

[b] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

[c] Computations performed at the 5% testing level, using the over-all Error Mean Square value for the determination of the Standard Error of a Treatment Mean. Specimens having the same group number are not significantly different from each other, but are significantly different from those in another group, provided that they are not contained in a subset (indicated by two or more numbers) of the groups being compared.

TABLE 5

Relative bacterial cleanability of glasses used in the dairy industry

Type of material and surface finish	Surface analyzer reading (μ" R.M.S.)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V	
		Average radio-activity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radio-activity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radio-activity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radio-activity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]	Average radio-activity after cleaning[a] *(counts/min)*	Grouping by Duncan's Multiple Range Test[b]
Type 7740 Borosilicate glass:											
Optically polished	0	1	*1*	2	*1*	19	*1*	42	*1*	14	*1*
Molded	0.4–0.7	2	*1*	2	*1*	28	*1*	42	*1*	—	—
Blue glass enamel lining PF (glazed)	0.3–2.5	3	*2*	2	*1*	24	*1*	44	*1*	—	—
Soda-lime milk bottle glass (Pristine)	0.5–0.8	3	*2*	2	*1*	26	*1*	46	*1*	13	*1*

[a] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

[b] Computations performed at the 5% testing level, using the over-all Error Mean Square value for the determination of the Standard Error of a Treatment Mean. Specimens having the same group number are not significantly different from each other, but are significantly different from those in another group, provided that they are not contained in a subset (indicated by two or more group numbers) of the groups being compared.

TABLE 6

Relative bacterial cleanability of various plastics

Type and manufacturers' specification number	Surface analyzer reading (μ" R.M.S.)	Experiment I		Experiment II		Experiment III		Experiment IV		Experiment V	
		Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]	Average radioactivity after cleaning[b] *(counts/min)*	Grouping by Duncan's Multiple Range Test[c]
Polyfluorocarbon											
FYTH[a]	0.4–0.8	5	*1*	9	*1*	23	*1*	35	*1*	—	—
KF[a]	0.4–0.8	3	*1*	11	*1*	22	*1*	35	*1*	22	*1*
1[a]	14–46	10	*2*	24	*2*	146	*2*	306	*2*	96	*2*
SPT (sponge)	130	—	—	607	*3*	723	*3*	1,259	*3*	687	*3*
Polyethylene											
DYNH[a]	0.2–0.4	3	*1*	9	*1*	27	*1*	37	*1*	10	*1*
1412-9-A[a]	22–30	6	*2*	8	*1*	54	*2*	63	*2*	46	*2*
Polyamide											
3001[a]	4–12	5	*1*	9	*1*	22	*1*	24	*1*	18	*1*
10001[a]	16–33	5	*1*	9	*1*	201	*2*	247	*2*	180	*2*
Polyvinyl chloride											
5960[a]	0.4–0.7	1	*1*	8	*2*	23	*1*	24	*1*	—	—
F-92[a]	1.0–2.0	3	*2*	8	*2*	33	*1*	32	*1*	—	—
1310[a]	0.1–0.2	6	*3*	5	*1*	22	*1*	24	*1*	19	*1*
6200[a]	2.5–2.8	6	*3*	11	*2*	26	*1*	28	*1*	—	—
Polyvinylidene chloride 548[a]	1.2–3.5	—	—	11	*1*	46	*1*	49	*1*	41	*1*
Polyester-Fiberglas											
HFL (laminated)	26	35	*1*	32	*1*	75	*1*	78	*1*	75	*1*
RHW-1-7 (laminated)	50	—	—	114	*2*	243	*2*	175	*2*	173	*2*
Polystyrene											
3002[a]	1.0–1.8	1	*1*	3	*1*	26	*1*	31	*1*	24	*1*
1150[a]	1.2–1.9	—	—	10	*1, 2*	33	*2*	45	*1, 2*	—	—
5001[a]	2.2–4.6	4	*2*	17	*2, 3*	51	*2, 3*	60	*2, 3*	—	—
5230[a]	30	—	—	15	*3*	82	*3*	95	*3*	75	*2*
Acetate-Butyrate											
1408-22-E[a]	0.2–0.5	4	*1*	8	*1*	25	*1*	25	*1*	21	*1*
Acrylic 140[a]	0.8–1.1	2	*1*	6	*1*	23	*1*	24	*1*	19	*1*

[a] Surface features impressed upon the specimen by the mold against which it was formed.

[b] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

[c] Computations performed at the 5% testing level, using the over-all Error Mean Square value for the determination of the Standard Error of a Treatment Mean. Specimens having the same group number are not significantly different from each other, but are significantly different from those in another group, provided that they are not contained in a subset (indicated by two or more group numbers) of the groups being compared.

TABLE 7

Relative bacterial cleanability of abraded[a] milk-contact surfaces

Type of material	Surface analyzer reading (μ" R.M.S.)		Average radioactivity after cleaning[b] (*counts/min*)				
			Experiment I	Experiment II	Experiment III	Experiment IV	Experiment V
	Before abrading	After abrading					
Stainless steel:							
Type 302	3	23	21	28	379	395	358
Nickel-base alloys:							
Inconel	55	30	18	25	393	499	271
Monel	5	54	73	118	470	625	287
Copper-base alloys:							
Casting Alloy LTA	36	27	—	27	254	837	208
Casting Alloy WKM	10	33	—	26	570	612	—
Wrought Sheet 18A	13	26	—	23	639	687	556
Aluminum alloys:							
Wrought Sheet 8	18	72	453	543	1,658	1,804	1,109
Glasses:							
Soda-lime milk bottle glass	0.7	50	27	31	162	209	111
Blue glass enamel lining material PF	1.4	180	84	73	326	572	297
Plastics:							
Polyfluorocarbon KF	0.6	65	19	18	956	980	912
1	25	76	21	29	158	216	117
Polyethylene 1412-9-A	25	57	26	38	999	1,092	942
Polyamide 3001	8	52	10	13	388	717	268
Polyvinyl Chloride 1310	0.2	48	13	18	1,319	1,606	992
Polystyrene 3002	1.5	56	21	25	1,284	1,576	1,107
Polyester-Fiberglas							
HFL	26	65	53	67	965	1,255	932
RHW-1-7	50	85	—	172	1,235	1,513	947
Acetate-Butyrate 1408-22-E	0.4	50	22	29	492	612	427
Acrylic 140	1.0	88	19	25	1,032	1,242	961

[a] Abraded by light rubbing with No. 1/2 Emery cloth for 10 min.

[b] Based on net counts/minute caused by bacteria remaining on the surfaces after cleaning; corrected for background, back-scatter, coincidence, and decay.

A striking difference was apparent between Polyamide Formulations 3001 and 10001. The former retained about one-tenth the amount of soil retained by the latter. Formulation 10001 was of a more spongy consistency than Formulation 3001 and, therefore, possessed a greater potential capacity for holding the bacterial soils.

In general, the abraded plastic specimens exhibited about five times the soil retention of the same materials as received. There were some notable exceptions to this average value.

Autoradiographic determinations. While the Geiger counting procedures were of value from a quantitative point of view, the autoradiographic techniques

group, provided that they are not contained in a subset (indicated by two or more group numbers) of the groups being compared.

provided valuable qualitative information. By this means, it was possble to ascertain the distribution of the bacteria on the various surfaces, and find out what kind of surface features harbored the organisms and protected them from the cleaning action of the chemicals and mechanical cleaning aids employed. Autoradiograms of several of the materials tested are shown (Figure 1). They represent the surfaces of the test discs (soiled with *Micrococcus pyogenes* var. *aureus* in 0.85% saline suspension) after being subjected to the standard cleaning procedure. All areas appearing white are radioactive bacteria.

Autoradiogram A is of stainless steel with a 2D finish. This surface is characterized by a random over-all coarseness. The radioactive bacteria are distributed in a random manner similar to the surface features. Some concentration at the grain boundaries is discernible.

Autoradiogram B represents a sand-blast finish. There is a high concentration of the organisms in the deeper pockets formed by the finishing operation on this specimen.

Autoradiogram C is of a spongy polyfluorocarbon plastic laminate used for

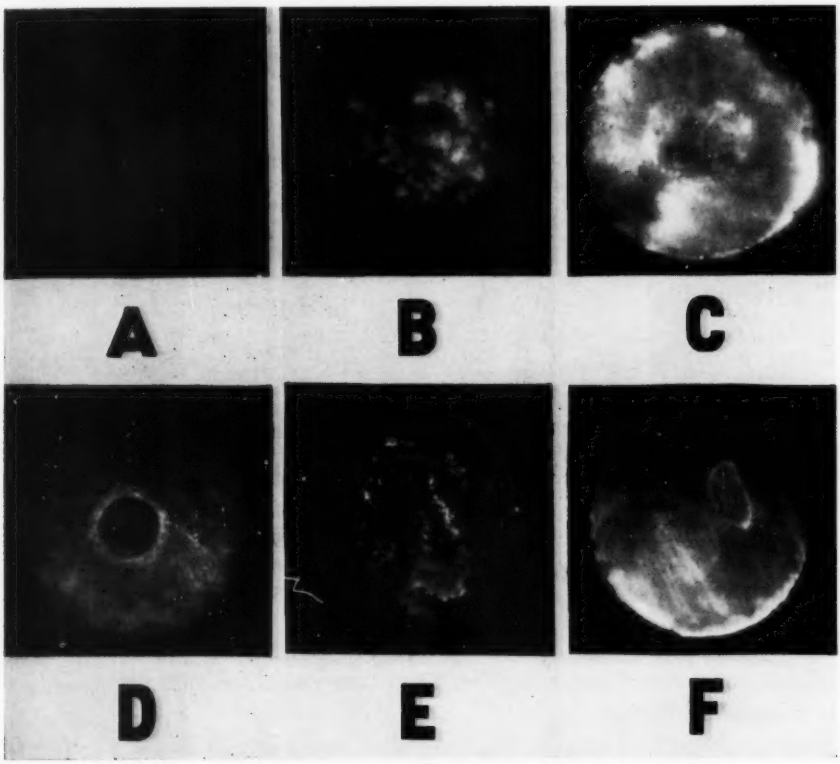

FIG. 1. Autoradiograms of test milk-contact surfaces after cleaning.

some types of gaskets. The number of bacteria retained by this highly porous surface is clearly evident. The greatest concentration of cells occurs where the porosity is the most pronounced.

Autoradiogram D represents Polystyrene 3002 abraded with emery cloth. The radioactive organisms are concentrated in the jagged grooves cut into the surface of this specimen. Other materials abraded in the same way showed a similar bacterial distribution.

Autoradiogram E shows a corroded specimen of copper-base Casting Alloy WKM, buff finish. The autoradiogram indicates that the corrosion products tend to retain bacteria; the areas of heaviest concentration of bacteria are where the corrosion has proceeded to the greatest extent.

Autoradiogram F is of a corroded specimen of copper-base Casting Alloy LTA, No. 4 polish finish. The general distribution of the radioactive bacteria can be seen to follow the lay of the attacked grooves of the surface finish.

Even as a qualitative tool, autoradiography has a potentially greater practical testing sensitivity than Geiger-counting techniques, because the X-ray film can be exposed to the radiations emitted from the source for a longer time than that normally used for a statistically significant count with a Geiger-counting assembly. With the proper calibration techniques, autoradiograms can yield quantitative data on relative concentrations of soil on different sections of a surface.

Agar submersion plate comparisons with radiological techniques. Direct submersion plates of representative cleaned specimens showed highly erratic results. The maximum deviation from the average of five colony determinations on these plates was of the order of 55%. In many cases, it was difficult to make out colonies against the specimen surfaces. In addition, copper-base alloys seemed to exhibit an inhibitory effect on the development of bacterial colonies. Generally, however, those specimens which counted high radiologically demonstrated more growth in agar than those which counted lower.

In contrast, when radiological counting was used on cleaned specimens, the deviation from the average of ten replicates selected at random was about 7%. This variation could be accounted for largely on the basis of differences likely to occur in the surface conditions of specimens selected at random.

DISCUSSION

In general, the experimental results show a greater retention of bacteria deposited upon a surface from a 0.85% saline suspension than from a homogenized whole milk suspension. This phenomenon might be explained by the nature of the carrier and by the extent of intimate contact between the bacterial cells and the surface in question. In a complex substance like milk, some constituents might act in a retaining capacity, while others might aid in the removal of the microorganisms. It is conceivable that the milk constituents likely to be fixed to the surfaces by heating were deposited to a greater extent in the surface features than the bacterial cells. Thus, most of the microorganisms were laid down upon, and surrounded by, these milk constituents which yielded readily

to the chemical and mechanical soil removal action of the cleaning procedure. The cells associated with those milk deposits were likewise removed with ease.

The saline carrier, on the other hand, would tend to permit a greater bacterial-surface interaction, and might, in addition, react with the material and/or bacteria to form a more cohesive bond between them. The need for further study is indicated by these data in the field of soil-substrate interaction.

It is evident from the data that a rough, jagged surface finish and/or a porous subsurface on any material will result in mechanical entrapment of soil particles, thereby reducing the cleanability of such surfaces. This is what might reasonably be expected on the basis of field experience. However, height and depth of surface features, as determined by R.M.S. measurements or similar means, is not the only criterion for estimating the soil-retaining capacity of a material. Rather, it is the nature of the surface and subsurface features (i.e., jagged vs. rounded peaks and valleys) which determines the physical surface effects of soil retention exhibited by a material. The chemical nature of the material probably exerts an influence on its cleanability, through chemical interaction, wettability, adsorption potentials, etc. Which effects or combination of effects predominate in a given situation can only be determined by controlled experimentation.

The phenomenon of soil buildup is indicated by the results of tests in which the discs were washed and then soiled for a second time. The counts on these discs after washing were about twice the counts after the first soiling and washing.

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CONGENITAL TAILLESSNESS IN CATTLE [1]

KEITH HUSTON AND STANLEY WEARDEN
Department of Dairy Husbandry and Statistical Laboratory, Kansas State College, Manhattan

SUMMARY

Taillessness, a rare congenital defect of cattle, has been observed in 25 animals sired by registered bulls of the Angus, Ayrshire, Guernsey, Hereford, Holstein, Jersey, Milking Shorthorn, and Shorthorn breeds. The dams of the tailless animals included descendants from one or more of these or from the Brown Swiss breeds.

In females, taillessness occasionally is accompanied by anterior and dorsal misplacement of the vulva and anus, concave rump, and stiffness of the rear legs. In males, the defect may be accompanied by misplaced or atretic anus, concave rump, and stiffness of the rear legs. Malformations of the vertebral column appear to be variable.

The defect was noted in more than twice as many females as males. Defective animals were from older dams, most of which had not been reported to have had malformed offspring previously.

Tailless animals seem unlikely to occur, on the average, more often than once in every 3,000 births, and may occur as infrequently as once in every 20,500 births. Although the defect seems to be of little significance, there is some slight evidence for the supposition of a hereditary basis for the defect in the Holstein breed.

Taillessness, one of the many congenital malformations of cattle, has been the subject of limited inquiry. Baldwin, according to Gilmore and Fechheimer *(4)*, found that taillessness was the second most common defect of calves observed by technicians of the Central Ohio Breeding Association. In a brief report on more than 50 tailless animals, Gilmore and Fechheimer *(4)* noted that some tailless animals had malformed or missing sacral vertebrae, weak loins, and high pinbones and genitalia. Fifty of the tailless animals were sired by 43 different bulls of eight major dairy and beef breeds. Close relatives of some tailless animals had shortened, kinky, or twisted tails. From results of breeding trials, these authors concluded that taillessness was neither a simple dominant nor a simple recessive trait. At least two other tailless animals have been reported: one in Switzerland *(10)* and one in Portuguese Guinea *(9)*.

The nature and occurrence of taillessness in 25 dairy and beef animals is described.

METHODS OF DATA COLLECTION

The data reported on these 25 animals form a part of a continually expanding collection of case histories of abnormal cattle. The primary interest in the establishment and maintenance of this collection has been to characterize the abnormalities of cattle, particularly those of cattle in artificially inseminated herds in this state. Hence, the disproportionately large number of case histories

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of artificially sired animals is simply a consequence of the methods and purpose of data collection and does not signify that abnormalities occur more frequently in artificially inseminated herds than in other herds.

Compilation of information on rare defects, such as taillessness, is hindered not only by the rarity of the defect but also by the limited recording and reporting of such defects. Consequently, information on such defects often must be accumulated over rather lengthy periods. The cases reported here include one animal born in 1936, one in the early 1940's, and 23 in the period from 1949 through 1956.

Reports of the birth of abnormal animals are received routinely from the Kansas Artificial Breeding Service Unit birth-reporting system; occasional reports are received from college representatives, farmers, magazines, and newspapers. Most reports originate in the Kansas Artificial Breeding Service Unit birth-reporting system which was initiated in 1950.

Under this reporting system, technicians report, on IBM mark-sense duplicate first-service breeding receipts, the births of normal and abnormal calves when the dams of the calves are inseminated first for their subsequent gestations. The technicians, and also the fieldmen of the unit, the state extension dairymen, and the county agents, whose primary interest is in reporting abnormal calves born in artificially inseminated herds, occasionally report other abnormal calves. Of the 25 animals, 19 were reported to the authors routinely through the Kansas Artificial Breeding Service Unit birth-reporting system. Six others were reported by fellow workers or had been described in newspapers or magazines. All but two of these six animals were born in Kansas.

Eleven of the tailless animals were inspected by college representatives; two additional animals were viewed from their photographs. The remaining twelve animals were inspected only by the technicians or the owners. Information not secured at inspection time was secured by correspondence. Since some information had to be secured in part from the owner's memory of the animals and their relatives, a conscious effort was made to word questionnaires so as to avoid biasing the replies.

RESULTS

Nature of malformation. The tail is absent (Figures 1 through 3). In females, the area of the rump immediately posterior to the end of the vertebral column is often deeply depressed, the depth and breadth of the depression varying with the fleshing of the animal. The median sacral crest often appears somewhat elevated and knobby (Figure 1). In some females, the anus and vulva appeared to be at sites dorsal and anterior to the normal (Figure 2), while in other females the anus and vulva were at normal sites (Figure 3). One female had only the vulval exterior orifice; the rectum emptied into the vagina. In the only male examined closely, 052A, the anus was in a normal location. Another tailless bull was reported as not having had an anus.

The absence of the tail seemingly causes the animal little or no discomfort. That the absence of the tail is of some slight physiological importance is evidenced

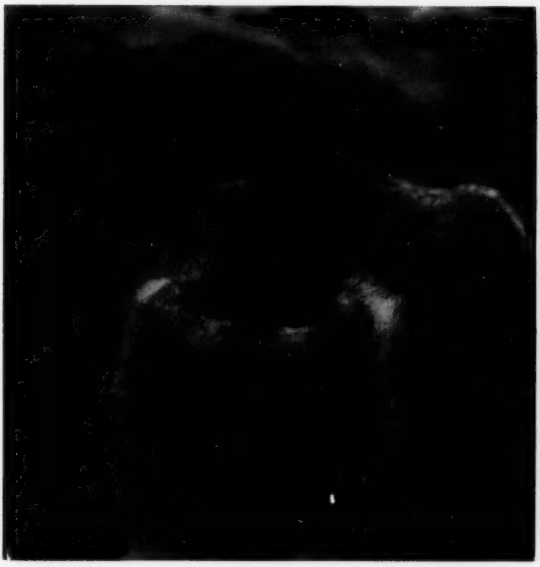

FIG. 1. Case No. 113, a purebred Jersey. Note rump depression, knobby sacral crest, and misplacement of vulva and anus. (Photo by E. P. Call.)

FIG. 2. Case No. 64A, a grade Shorthorn. Note misplacement of anus.

FIG. 3. Case No. 60A, an Angus-Holstein crossbred. Note rump depression and normal position of anus and vulva.

by post-mortem findings in the tailless heifer with the vulvovaginal anus. This heifer had a small forked stick in the soft tissue between the end of the vertebral column and the orifice of the vulva.

The hind leg movement of tailless animals ranges from normal to a shuffling gait on the walk, and an awkward, seemingly stiff-legged hopping motion on the run. This abnormal gait probably is a result of anatomical changes, of which only the osteological changes in two specimens can be reported.

The posterior ends of the vertebral columns of two 2-yr.-old animals were obtained after slaughter. A strip about 3 in. wide was sawed from the dorsal midlines of the animals. The transverse processes of the lumbar vertebrae, the wings of the sacrum, and the edges of the lateral sacral crests were not obtained in this process.

The specimen from the heifer 62A consisted of the bony structures posterior to the last lumbar vertebra and did not include the body of the first sacral vertebra. All sacral vertebrae appeared to have been present, although the third, fourth, and fifth deviated from the median plane to the right. The fifth was incompletely developed. Several vertebral remnants, presumably coccygeal, were present (Figure 4).

The specimen from the bull 056A consisted of the bony structures posterior to the first lumbar vertebra. The third and fourth lumbar vertebrae were fused, except in the dorsal halves of their spinous processes. The sacrum was abnormal and in two large parts: a separate first sacral vertebra and an irregularly fused mass posterior to it. The mass appeared to have been made up of all or parts of the four other sacral vertebrae. No coccygeal vertebrae were evident.

Sex and parentage of tailless animals. Of the 24 animals of known sex, 17 were females and 7 males (Table 1). This sex ratio departs significantly

FIG. 4. Posterior end of vertebral column of Case No. 62A (AB). Note presence of five sacral vertebrae (Nos. 1 through 5). Note coccygeal vertebra (C) and remnants of coccygeal vertebrae. (Specimen prepared by H. T. Gier, Department of Zoology.)

($\chi^2 = 4.66$, 1 d. f., $P < 0.05$) from the 53 : 47 ratio of females to males found among 10,228 births reported through the Kansas Artificial Breeding Service Unit birth-reporting system.

Tailless animals were sired by registered bulls of the Ayrshire, Guernsey, Holstein, Jersey, Milking Shorthorn, Shorthorn, Hereford, and Angus breeds. The dams of the tailless animals included descendants from one or more of these or the Brown Swiss breeds.

Age of parents and older maternal sibling history. The ages of the dams at the time the tailless animals were dropped ranged from about 3 yr. to about 16 yr. (Table 2). The birth dates of some dams were not known exactly; their ages were estimated by the owners as being within a range encompassing at most 2 yr. Considering only the least of such age estimates, the mean age of all dams was about 7 yr., 4 mo. There being no reason to suppose that the ages at which cows drop tailless offspring should differ from the ages at which they drop tailed offspring, the ages of the dams were arranged in order from greatest to least and compared with the usual distribution of calving ages of many cows. It seemed that the chances of cows dropping a tailless calf increased as the cows aged.

Before testing this, the dams were classified as either of beef or of dairy (including dual-purpose) origin. The five beef dams, dams of 056A, 116, 30, 115, and 93, were assumed to have been selected randomly from a large num-

TABLE 1

Sex, parentage, and other facts pertaining to tailless cattle

	Case number	Sex	Inbreeding coefficient	Other descriptive facts	Sire's breed and number	Dam's breed
			(F)			
1)	34 AB	F	Unknown	Normal walk	R.[a] Holstein (H-21)	G.[a] Holstein
2)	118 AB	F	0.00		R. Milking Shorthorn (M-2)	R. Milking Shorthorn
3)	38	M	0.00		R. Ayrshire	R. Ayrshire
4)	056 A	M	0.16	Normal walk	R. Shorthorn	R. Shorthorn
5)	116	F	.....	Anus high. Rump sunken	R. Hereford	G. Shorthorn
6)	89 ABS	F	Unknown		R. Holstein	G. Holstein
7)	396FF-OS	F	Unknown		R. Guernsey	R. Guernsey
8)	36 AB	M	Unknown	Rabbit walk. Stiff hindquarters	R. Holstein (H-16)	G. Holstein
9)	30	F	Unknown	Tail off at thurls. Stiff; moved with hop	R. Hereford	G. Hereford
10)	115	F	0.00	Normal movement	R. Shorthorn	R. Shorthorn
11)	114 AB	F	Unknown		R. Guernsey (G-11)	G. Guernsey
12)	FEE-5AB	M	Unknown	Normal in other respects	R. Holstein (H-7)	Holstein × Swiss
13)	62 A(AB)	F	Unknown	No exterior anus. Rectum emptied into vagina	R. Milking Shorthorn (M-8)	G. Milking Shorthorn
14)	117 AB	M	Unknown	Atresia Ani. Breech presentation	R. Holstein (H-16)	Unknown
15)	84 AB	M	Unknown		R. Holstein (H-16)	Unknown
16)	93	F	Unknown		R. Angus	Angus
17)	FEE-8AB	F	0.00	Anus anterior. Vulva at normal anal level	R. Milking Shorthorn (M-1)	R. Milking Shorthorn
18)	113	F	0.00	Anus high	R. Jersey	R. Jersey
19)	60 A	F	.....		Angus	R. Holstein
20)	112 AB	M	Unknown		R. Milking Shorthorn (M-4)	Unknown
21)	57 AB	F	Unknown	Normal in other respects	R. Holstein	G. Holstein
22)	61 A	F	Unknown	Normal movement. Right eye opaque	R. Milking Shorthorn (M-7)	Guernsey × Shorthorn
23)	105 AB	F	Unknown		R. Holstein (H-7)	G. Holstein
24)	111	Unknown	Unknown	No anus	Unknown	Holstein
25)	110-OS	F	Unknown		Unknown	Unknown

[a] R—registered; G—grade.

TABLE 2

Age of parents and older maternal sibling number and history of tailless cattle

	Case number	Age[a] of parents at birth of tailless animal		Number and history of older maternal siblings of tailless cattle
		Dam	Sire	
		(yr.)		
1)	34 AB	16[b]	8	All normal
2)	118 AB	13	10	8 normal
3)	38	11	7	8: 7 normal, 1 two-headed
4)	056 A	10	8	6 normal
5)	116	9	Unknown	6 normal
6)	89 ABS	9[b]	9	Unknown
7)	396 FF-OS	9	Unknown	6: 5 alive, 1 still-born
8)	36 AB	8[b]	9	Unknown
9)	30	8[b]	Unknown	5 or 6 normal
10)	115	7	4[b]	Unknown
11)	114 AB	7	11	5 normal
12)	FEE-5AB	7	9	5 normal males
13)	62 A (AB)	6[b]	8	3 normal
14)	117 AB	6[b]	9[b]	Unknown
15)	84 AB	6	10	Unknown
16)	93	6[b]	7[b]	3 normal
17)	FEE-8 AB	5	11	2 normal
18)	113	5[b]	4[b]	3 normal
19)	60 A	4	Unknown	All normal
20)	112 AB	4[b]	9[b]	1 normal
21)	57 AB	3[b]	Unknown	None
22)	62 A	3[b]	8	Normal bull. Dwarf younger sib.
23)	105 AB	Unknown	13	Unknown
24)	111	Unknown	Unknown	Unknown
25)	110-OS	Unknown	Unknown	Unknown

[a] Age to nearest whole year.
[b] Estimated by owner or from owner's records.

ber of typical Kansas beef cows. From information furnished by Smith *(8)*, these beef cows may be classified in groups calving at various ages, each calving age group constituting the following percentage of the total cows: three and fewer years, 14.5; four and fewer years, 29.0; five and fewer years, 41.0; six and fewer years, 52.0; seven and fewer years, 63.0; eight and fewer years, 73.0; nine and fewer years, 83.0; ten and fewer years, 92.0; and all ages, 100. All 17 other dams were assumed to have been selected randomly from a large number of typical Kansas dairy cows. From Kansas DHIA data reported by Asdell *(1)*, these Kansas dairy cows may be classified in calving age groups which make up the following percentages of the total: three and fewer years, 24.3; four and fewer years, 41.9; five and fewer years, 56.0; six and fewer years, 67.5; seven and fewer years, 77.2; eight and fewer years, 84.5; nine and fewer years, 90.2; ten and fewer years, 93.9; and all ages, 100.

The beef dams, mean age of about 8 yr., seem to be from a group of cows which calve at ages greater than those typical of Kansas beef cows (one-tailed Kolmogorov-Smirnov test; $c = 0.52$, $P\{c > 0.52\} = 0.04$) *(2, 5, 7)*. The dairy and dual-purpose dams, mean age of about 7 yr., 2 mo., also seem to be from a group of cows which calve at ages greater than those typical of Kansas dairy cows (one-tailed Kolmogorov-Smirnov; $c = 0.32$, $P\{c > 0.32\} = 0.02$).

The sires of the tailless-animals averaged 8 yr., 11 mo. in age at the time

these tailless progeny were born (Table 2). This average age might seem somewhat advanced. However, since bulls owned by artificial breeding organizations sired 14 of the tailless animals, and such bulls usually are older than bulls in natural service, a somewhat higher average age should be expected. Although there seemed to be no reason to suspect that aging of sires might be of significance in the etiology of taillessness, this possibility was examined. Among the 13 animals sired artificially by bulls of known age, 12 were sired by bulls owned by the Kansas Artificial Breeding Service Unit. The ages of these bulls when tailless animals were conceived averaged 8 yr., 7 mo. No significant differences were found in the relative frequencies with which tailless progeny occurred among the progeny which these bulls sired at different ages (two-tailed Kolmogorov-Smirnov; $D = 0.23$, $P > 0.20$).

The tailless animals collectively had at least 62 older maternal siblings. Among these 62 or more older maternal siblings were a two-headed calf and a stillborn calf (Table 2). The odds of these two abnormal calves appearing among 62 births were estimated from other Kansas data. In about 37,000 reported births in Kansas DHIA herds, 46.4 stillbirths were noted among every 1,000 births. In about 5,200 births reported through the Kansas Artificial Breeding Service Unit birth-reporting system, 1.7 calves with disfiguring abnormalities were noted among every 1,000 births. Based on these frequencies, one or more stillbirths should be found in 95 out of every 100 samples of 62 births, and one or more disfigured calves should be found in 10 out of every 100 samples of 62 births. Two or more calves which were stillborn or disfigured should be found in from 79 to 81 of every 100 samples of 62 births. Thus, neither of these events is so rare as to suggest that explanation of their occurrence, singly or jointly, necessitates the supposition of a predisposition to defectiveness among older maternal siblings of tailless animals.

Evidence of the effects of heredity differences. Evidence of a genetic basis for this defect was sought in differences in the frequency of occurrence of tailless births among breeds and among certain subgroups within breeds. Information on all 25 tailless animals could not be used, either because parentage was incompletely recorded, or because the groups from which these animals came could not be specified.

Among the 25 tailless animals, there were only 12 which resulted from matings to bulls in the Kansas Artificial Breeding Service Unit. These 12 animals represent a small fraction of all births occurring during the period from 1950 through 1956 and resulting from inseminations made by technicians of the Unit. The total number of artificially sired animals can be estimated nearly maximally as being equal to the number of first services throughout all but the last 9 mo. of this period. This number was 246,616.

This number probably does not correspond exactly with the more desirable but unobtainable number of births. A small percentage of the first-service cows, perhaps not more than 1–2% according to Asdell's figures *(1)*, does not conceive after varying numbers of services, and is slaughtered. Another, probably much

larger group, though at the most probably not greater than 20% of the first-service cows, conceives, but the pregnancies are terminated by slaughter of animals which have lost their economic utility. A third group consists of pregnant animals sold from the reporting area.

With these limitations in precision, the nearly minimal frequency of tailless births can be estimated at about one in 20,500 births, assuming, of course, that the 12 tailless animals were the only tailless animals resulting from the inseminations made during this period.

If, as seems likely, more than 12 tailless animals resulted from these matings, the frequency of tailless births may be greater than the minimal estimate. The 12 animals were reported through the Kansas Artificial Breeding Service Unit birth-reporting system. The births reported through this system represent about 15% of the corresponding antecedent first services. Assuming that these 12 animals were the only tailless animals resulting from only this 15% of the inseminations, the nearly maximal frequency of tailless births can be estimated at about one in 3,000 births.

The difference between these two estimates seems quite unlikely to have arisen simply through chance variation in sampling from a large group of animals containing some definite proportion of tailless animals. Acceptance of the maximal estimate as being correct requires accounting for some 78 tailless animals not being reported. On the one hand, this number may seem quite large, although it could result just from the failure of persons in each county in the state to report a single tailless calf sometime during the 5 yr. period. On the other hand, because of the relative rarity of the defect, and its absurd and easily identified but trifling effect on the appearance of the animal, its occurrence should be reported readily by most farmers who might not be so willing to report more grotesquely defective calves. More precise estimates were desired, but there seemed to be no practical way of obtaining them.

In a similar fashion, and with the same limitations to accuracy, the breed origins of the 12 tailless animals were examined for evidence of differences among breeds in the frequency of occurrence of tailless births (Table 3). Assuming that the maximal frequency of about one tailless birth in every 3,000 births is the true frequency, the differences observed in the frequencies of tailless births among breeds could have come about through random variation in sampling

TABLE 3

Frequency of tailless births in six breeds of cattle

Breed	Tailless animals	First services	Estimates of frequency of tailless births	
			Minimal	Maximal
	(No.)		(/100,000 births)	(/100,000 births)
Holstein	6	136,356	6.6	29.3
Milking Shorthorn	5	35,393	16.1	96.2
Guernsey	1	31,814	3.2	20.9
Jersey	0	16,007	0.0	0.0
Brown Swiss	0	15,385	0.0	0.0
Ayrshire	0	10,571	0.0	0.0
All breeds	12	246,616	6.9	32.6

($0.70 > P' > 0.50$; breed probabilities of equal or greater number of tailless animals estimated by central-limit theorem corrected for continuity, assumed independent, and compounded by Fisher's method)*(3)*. Likewise, the differences observed in the frequencies of tailless births among breeds could have come about through random variation in sampling from a large group of animals in which the minimal frequency, one tailless animal in 20,500 births, is assumed to be the true frequency ($0.70 > P' > 0.50$). Thus, it seems unnecessary to assume from these data that the differences among breeds exist in the frequency of occurrence of tailless births.

The importance, if any, of heredity in causing taillessness within a breed had to be adduced from the occurrence of a greater degree of inbreeding in the pedigrees of tailless animals than commonly exists in that breed, or the existence of a greater degree of relationship among the tailless animals than commonly exists in a randomly selected group in the breed involved.

The only animal known to have resulted from a markedly inbred mating was 056A (Table 2). This bull was born in the inbred Kansas State College beef Shorthorn herd, the only tailless animal produced from 36 such matings.

The relationship among the tailless animals of each breed was measured with Wright's coefficient, R, from pedigrees extended to include ancestors born prior to 1935. Most of the dams of the tailless animals were grades and of unrecorded ancestry. Hence, most of the relationship coefficients necessarily were based on ancestry common only to the sires of the tailless calves.

The two Guernsey animals were unrelated, as were the two beef Shorthorn animals. The eight animals by registered Holstein bulls were all artificially sired; six by bulls belonging to the Kansas Artificial Breeding Service Unit; one, 89 ABS, by a bull in another stud operating in Kansas; and one, 57AB, by an unidentified bull in a stud in an adjacent state. Neither 89ABS nor the dam of 60A was related to each other or to the tailless animals sired by bulls of the Kansas Artificial Breeding Service Unit.

The inter se relationship coefficients of the tailless Holstein animals sired by bulls of the Kansas Artificial Breeding Service Unit were compared to the inter se relationship coefficients of all artificially sired progeny of these bulls. This comparison also was made for the Milking Shorthorn animals, all of which were sired by bulls of the stud (Table 4). If there were a common hereditary basis for the tailless trait, tailless animals of a breed should be more closely related than animals selected randomly from the entire breed group. The Hol-

TABLE 4

Average inter se relationship of all progeny and of all tailless progeny sired by Holstein and Milking Shorthorn bulls in the Kansas Artificial Breeding Service Unit

Breed	Inter se relationship coefficients: All progeny: No.	Av.	Tailless progeny: No.	Av.
		(%)		*(%)*
Holstein	9,334,581,408	2.31	15	10.81
Milking Shorthorn	626,141,281	3.08	10	0.31

stein tailless animals seem to have come from a group of animals which was more closely related than the average relationship among all artificially sired Holsteins (one-tailed Kolmogorov-Smirnov, $c = 0.57$, $P\{c > 0.57\} < 0.001$). The differences in the Milking Shorthorn breed could have arisen through random sampling variation (two-tailed Kolmogorov-Smirnov, $D = 0.14$, $P > 0.20$).

The last bit of evidence concerning the effects of heredity is found in the fact that a single-first-cousin of animal 62A was reported to have been tailless.

DISCUSSION

In addition to the cursory but requisite anatomical and physiological description of tailless animals, an attempt has been made to interpret other data on 25 tailless animals, in terms of working hypotheses about etiologically contributory factors. Only three require additional discussion.

The significantly disparate sex ratio observed among the tailless animals may reflect a greater propensity to taillessness among female offspring than among male offspring. Or, it may have resulted from a rare random sampling event, or from biased sampling. The birth of a malformed female calf, at least in the dairy breeds, is of considerably more economic significance to the owners than is the birth of a malformed male calf. Thus, births of markedly malformed females might be reported more completely than the births of markedly malformed male calves.

The preponderance of mature, parous dams, clearly out of proportion to their numbers in the cow herds in this state, may be indicative of an association between taillessness and either advancing maternal age or increasing number of calvings, or both. It is unlikely that in cattle the effects of increasing number of calvings can be separated from those of maternal aging. Similar effects previously have been shown to exist in humans, wherein the incidence of certain congenital malformations seems to increase with both birth order and maternal age *(6)*.

The evidence relative to a hereditary basis for the defect is quite limited in these data. Only two breeds were represented by more than two tailless animals. Of these two breeds, only in the Holstein was there a hint that hereditary differences in some way might be responsible for the occurrence of taillessness.

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HEREDITARY CONGENITAL ATAXIA IN HOLSTEIN-FRIESIAN CALVES [1]

K. R. JOHNSON, D. L. FOURT, AND R. H. ROSS
Department of Dairy Husbandry
AND
J. W. BAILEY
Department of Veterinary Science
University of Idaho, Moscow

SUMMARY

A total of eight calves showing spastic symptoms was found in two related herds of Holstein-Friesian cattle over a 2-yr. period. The symptoms were those of incoordination or ataxia, a failure of synergic muscle groups to act harmoniously. The symptoms were first apparent at about 6 wk. of age and were similar in all cases. The disease is not sex-linked, since calves of both sexes were affected. An analysis of the pedigrees of the calves revealed one common male ancestor. This bull appeared in the pedigrees of both the sire and dam of all the affected calves. The ataxia is postulated to be inherited as an autosomal recessive. The occurrence of ataxic calves ceased when bulls known to be carriers were removed from the herd. The anatomical basis for the condition was neuronal degeneration and cerebellar hypoplasia.

The economic importance of lethals in cattle can be great. The average cow produces only a few offspring in a lifetime. Lethals preventing the perpetuation of a particular line of breeding containing genes of desired characters could result in great economic loss. The total loss due to inherited lethals is not known. However, they would constitute a portion of the calves born dead. This number has been tabulated by Linn *(7)* at between 5 and 6% in approximately 25,000 calves born to Kansas D.H.I.A. cows.

A realization of the importance of the problem of inherited lethals in dairy cattle has been accentuated by the rapid growth of artificial breeding.

Gilmore *(3)* lists 28 lethal characters in cattle, two of which are congenital spasms and cerebellar hypoplasia.

Saunders *et al. (8)* reported nervous symptoms in 23 calves born in a purebred Jersey herd over a 15-yr. period. The clinical symptoms were those of incoordination. The symptoms were present at birth in some of the calves and appeared during the first week or two of life in the others. Although such calves could nurse and had good appetites, all died within a few weeks. The anatomical basis for the symptoms was an anomaly of the cerebellum and midbrain.

Congenital spasms were reported by Gregory *et al. (4)* to occur as a recessive lethal condition in a Jersey herd. Affected calves exhibited intermittent spasmodic movements of the head and neck, usually in a vertical plane. When the animals were forced to stand, spasms in both front and hind legs hampered standing or walking. Aside from the spasms, such calves, up to a few days of age, appeared

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to be normal and vigorous and to have good appetites. They died, however, within a few weeks after birth.

A familial cerebellar ataxia in purebred Hereford calves in England was reported by Innes *et al. (6)*. The syndrome was apparent at birth and was clinically similar to that reported in Jersey calves by Saunders *et al. (8)*. The cerebellum of affected calves was abnormally small, and was tough in consistency. The lesion was interpreted histologically as a cortical hypoplasia. In two of these herds, replacement of the bulls resulted in a cessation of the disease. Hereford bulls that had sired ataxic calves from Hereford cows sired only normal offspring from Shorthorn cows. The disease was thought to be due to a lethal recessive gene that was restricted to the Hereford breed.

Congenital spasms were reported by Cole *(2)* as a recessive lethal condition in Holstein-Friesian calves. A 3-mo.-old Holstein-Friesian bull calf with congenital cerebellar hypoplasia was found in the Denver, Colorado, Stockyards by Anderson and Davis *(1)*. Congenital hereditary cerebellar ataxia has been observed in various breeds of sheep in Great Britain and Canada by Innes and MacNaughton *(5)*.

Some diseases with comparable clinical symptoms have been presented in this review of literature for purpose of comparison. However, none of the calves discussed in the reviews were so long in developing the disease or survived so long afterwards as the calves in this study.

MATERIALS AND METHODS

Eight calves with extreme spasmodic movements were observed (over a 2-yr. period) in two related herds of Holstein-Friesian cattle. The eight calves, six females and two males, of which one male and one female were full brother and sister, all exhibited the same clinical symptoms. The age at which the symptoms were first observed varied from 6 wk. to 5 mo. The ataxia was barely noticeable when first observed and grew progressively worse until the calves were destroyed. Since almost all of the bull calves from the two herds were sold at three days of age, it is not known how many of these calves would have developed the disease.

The clinical symptoms for all the affected calves were those of incoordination or ataxia, a failure of synergic muscle groups to act harmoniously. The calves exhibited intermittent spasmodic movements of the head and neck, usually in a vertical plane. When the animals were forced to stand, spasms in both front and hind legs hampered standing or walking. Animals stood with all four legs braced in a straddled position and, when attempting to move, showed marked incoordination (Figure 1). An attempt to walk resulted in a fall, usually backward (Figure 2). Noise and shock neither initiated nor intensified the spasms. Aside from the spasms, such calves appeared to be normal and vigorous and to have good appetites.

RESULTS

Five calves, four female and one male, that had become spastic and developed ataxia were autopsied at 6–13 mo. of age by the Department of Veterinary

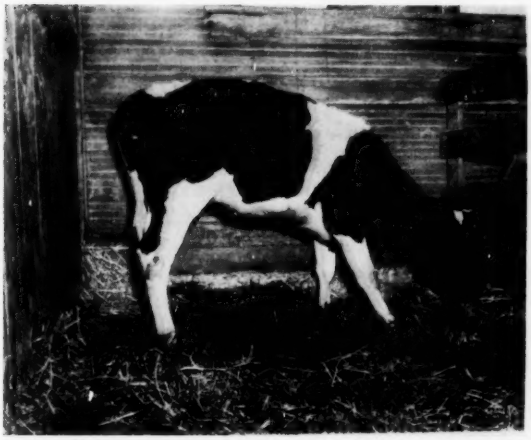

FIG. 1. Ataxic calf standing with legs braced in a straddled position.

Pathology, State College of Washington. The case history and autopsy results of the five calves were so similar that there is little doubt that the calves had the same disease. Since the cases were so similar, an autopsy report is given for the oldest calf observed.

This calf was born in February and the spastic condition first noted in July. The calf, until that time, had appeared to be normal, with a good appetite, and had a normal rate of gain. The spastic condition developed into ataxia in about 6 wk. The ataxia progressed until the calf could no longer stand after being helped to its feet. Autopsy was performed in March at 13 mo. of age. The results of the autopsy were: main thoracic and abdominal organs normal with the exception of the kidney, where small, pale, wedge-shaped areas were

FIG. 2. Ataxic calf falling backward when attempting to walk.

observed in the cortex. Microscopically, these were found to be focal areas of interstitial nephritis. The brain appeared small and was found to weigh 301.3 g. The brain of a normal calf of nearly the same size examined on the same day weighed 450 g. The convolutions of the cerebellum appeared small and very closely packed together and, in at least two instances, they projected upward into the dura in a papillary fashion. The dura appeared to be thickened over the area of the cerebellum and was found to have a slight degree of fibrosis.

The microscopic examination of the brain revealed a mild neuronal degeneration in the thalamus, the cerebellum, and the cerebrum. There was moderate satellitosis and gliosis in all areas examined, and the cerebellum showed a conspicuous degeneration of the Purkinje cells, with a reduction in the number of cells in the granular layer. There was a moderate diffused gliosis and a slight neuronal degeneration in the medulla. Sections of the spinal cord appeared normal.

There appeared to be some distortion of the cranial cavity, with a reduction in the space available for the cerebellum and the medulla. The tentorium, which tends to separate the medulla and cerebellum from the cerebrum, was very small and poorly developed. There is some possibility that the failure of the development of the bones of the cranial cavity, or some distortion in the direction of development of the bones, may have caused increased pressure on the brain and interfered with its proper development.

The pedigrees of the affected calves were studied, to determine whether or not the condition could have been inherited. One male was found to appear in the pedigree of both the sire and dam of all the affected calves (Figure 3). Calves 1 and 2 were full brother and sister. They trace back to this common male ancestor five generations through their sire's pedigree and four generations through their dam's pedigree. Calf 3 is four generations from this ancestor through both the sire's and the dam's pedigree. Calves 4 and 8 are four generations away through the sire's pedigree and five generations away through the

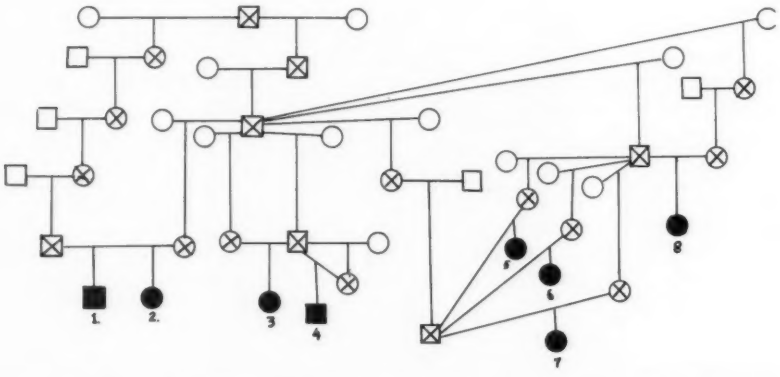

FIG. 3. Pedigree showing inheritance of congenital ataxia in Holstein-Friesian calves.

dam's pedigree. Calves 5, 6, and 7 are five generations away through both their sires' and dams' pedigrees. Although all the animals listed as known carriers in Figure 3 did not produce ataxic calves, they are in the direct line of descent from the one common ancestor. Therefore, it was assumed that they were carrier animals.

None of the parents of the ataxic calves showed any of the nervous symptoms. Since the affected offspring were born from phenotypically normal parents, the disease, if inherited, would have to be due to a recessive gene. As both sexes were included among the affected calves, the condition could not be sex-linked. No graduation in the symptoms was observed. The calves either had all the symptoms or none, thus eliminating multiple factors, unless the disease was due to threshold-type inheritance. It was postulated that the ataxia was conditioned by an autosomal recessive gene. The breeding of heterozygous carriers would then result in affected calves whenever a calf was homozygous for this disease.

A planned inbreeding program was carried out in which a known carrier bull was bred to his daughters and daughters of his half-brother, including three known carrier females. One of three resulting calves was affected.

The occurrence of ataxic calves has ceased since males and females known to be carriers of the disease have been removed from the herds.

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EFFECT OF OXYTOCIN AND EPINEPHRINE ON THE CONCEPTION RATE OF COWS

R. L. HAYS, N. L. VAN DEMARK, AND E. E. ORMISTON
Department of Dairy Science, University of Illinois, Urbana

SUMMARY

The effect on conception rate of injecting oxytocin just after, or epinephrine just before, natural mating and artificial insemination was tested with three groups of normal cows and two groups of "hard-to-settle" cows. Intravenous injections of 15 I.U. oxytocin within 5 min. after natural mating brought about a significant increase in conception in one group of normal cows (56 vs. 84% actual calvings). A similar trend (not statistically significant) was found with a second group of normal cows (48 vs. 56%) and with both groups of hard-to-settle cows (39 vs. 52% and 21 vs. 58%). An intravenous injection of 2 ml. of a 1/1000 solution of epinephrine within 5 min. before natural mating in one experiment resulted in an increase in the per cent of cows that conceived (controls 48 vs. treated 58%, not significant). The increase was similar to that shown by oxytocin injections. Neither oxytocin nor epinephrine treatment significantly affected conception rate in one group of artificially inseminated cows as compared to controls (controls 73 vs. oxytocin 67 vs. epinephrine 68%, 60- to 90-day nonreturns to service).

The finding that sperm were transported to the ovarian end of the oviduct of the cow in 3 min. or less *(9)* led to experiments designed to discover the cause of this rapid transport of sperm. It was shown that uterine activity was markedly increased by the various stimuli of artificial insemination and of natural mating *(7)*. Furthermore, this uterine activity was caused by oxytocin or an oxytocin-like substance released as a result of the stimuli of mating *(4)* or mechanical stimulation of the uterus *(1)*. Intravenous injections of oxytocin produced uterine activity similar to that caused by artificial insemination or natural mating *(3)*. Also, perfusion of oxytocin into excised reproductive tracts of cows was largely responsible for the transport of sperm from the mid-cervix through the uterus and into the oviducts in a matter of minutes *(8)*. Epinephrine has been shown to inhibit the action of oxytocin on the uterus *(3)*.

From the facts that have been established regarding oxytocin release at mating and artificial insemination, and the effects of oxytocin and epinephrine on uterine motility, one would suspect that increased release of endogenous supplies of these hormones or injections of them might affect conception rates. Besancon *et al. (2)* found that massage of the uterus and cervix for 1–3 min. at the time of artificial insemination resulted in a small (not statistically significant) increase in conception rate over cows not given the extra massaging. Tjupic *(6)* reported that 78% of 51 cows that received uterine massage conceived, as compared to 59% of 80 control cows. This difference was significant. Pounden and Firebaugh *(5)* found that cows rated as unusually complacent or unusually nervous at the time of artificial insemination showed a lower conception rate than cows rated as moderately excited at the time of service. Thus, field observations tend to bear out the suppositions made from laboratory findings.

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In view of this, experiments were conducted to determine the effect of supplemental oxytocin and epinephrine (by intravenous injections), given at the time of natural mating or of artificial insemination, on the conception rate of dairy cows.

METHODS

The cows used had normal reproductive histories except for those in the hard-to-settle category. In the first experiment, the cows to be bred naturally to a Guernsey or to a Holstein bull were divided at random into two groups. One group received an intravenous injection of 15 I.U. of oxytocin within 5 min. after mating and the other group was untreated. If a cow failed to conceive to the first service, it was assigned for the second service to the opposite group from that to which it was originally randomly assigned. Thus, any unrecognized sterile cows had an equal effect on the conception rate in both groups. Only first and second services were used and conception rate was determined by actual calving.

In the second experiment, also involving natural mating, the cows and heifers to be bred to each bull were divided at random into three groups. Group One received an intravenous injection of 15 I.U of oxytocin within 5 min. after mating; Group Two received an intravenous injection of 2 ml. of a 1/1,000 solution of epinephrine hydrochloride within 5 min. before mating, and Group Three served as controls. Cows failing to conceive at the first service again were randomly assigned to another group for the second service. One Ayrshire, one Brown Swiss, and three Holstein bulls were used. Conception rate was again by actual calving.

The third experiment was conducted with artificially inseminated cows in cooperation with the Northern Illinois Breeding Cooperative. The experimental groupings were the same as in Experiment Two. The cows were randomly assigned to one of three groups (control, oxytocin-treated, or epinephrine-treated) and the results were determined on the basis of the 60- to 90-day nonreturn to service rate for the first and second services only.

In conjunction with Experiments Two and Three, the effect of intravenous injections of oxytocin on the conception rate of hard-to-settle cows was tested. The cows designated as hard-to-settle had failed to conceive from three previous services. These cows were placed at random into a control group that received no treatment, or into a group that received 15 I.U. of oxytocin within 5 min. after being bred naturally or artificially.

RESULTS AND DISCUSSION

The conception rates obtained in each of the three experiments, excluding the hard-to-settle cows, are shown in Table 1. The oxytocin-treated cows had increases of similar magnitude in conception rate in four of the five groups. Only in Experiment One was this difference in percentage of cows calving significant as indicated by the chi-square test. These increases in conception from the oxytocin treatment suggest that some cows are deficient in the release of

TABLE 1

Effect of injecting oxytocin and epinephrine at the time of natural mating or artificial insemination on the conception rate of dairy cows

	Control	Oxytocin	Epinephrine
	Experiment 1—Natural Mating		
No. cows	25	19	....
Cows calving	14	16	....
Per cent calving	56	84	....
	Experiment 2—Natural Mating		
No. cows	54	54	50
Cows calving	26	30	29
Per cent calving	48	56	58
	Experiment 3—Artificial Insemination		
No. cows	85	51	47
60- to 90-day nonreturn[a]	62	34	32
Per cent NR	73	67	68

[a] Cows not returning to estrus within 60 to 90 days of first service.

oxytocin at the time of natural mating. This might well result in reduced sperm transport in these cows *(8)*; fewer sperm reaching the site of fertilization could account for the lower conception rate. Further tests must be made to determine whether there is a deficiency in the release of oxytocin, due to a high threshold for stimulation in some cows, or faulty or incomplete stimulation on the part of the bull. Further work is needed also to establish the optimal dosage of oxytocin.

From the recorded effects of epinephrine on the bovine uterus *(3, 7)*, one would expect that epinephrine injections within 5 min. before natural mating would reduce uterine activity and in turn reduce conception rate. This did not occur in Experiment Two (Table 1) for, instead of the anticipated decrease, an increase in conception over the control group resulted from epinephrine injections. The increase was not significant.

The differences in conception (based on 60- to 90-day nonreturns) in the control, oxytocin-treated, and epinephrine-treated groups of cows artificially inseminated (see Table 1) were small and were not significant. Perhaps the inseminator that is capable of obtaining a conception rate near 70% (based on 60- to 90-day nonreturns) has already produced maximum stimulation and release of oxytocin.

No clear explanation is known for the fact that epinephrine did not lower conception either in natural mating or in artificial insemination. However, it has since been found that the dosage of epinephrine used in these experiments will not eliminate the pronounced uterine contractions that occur right after natural mating. Further, it is known that the effects of epinephrine are of short duration *(3)*. It seems possible that reduced uterine tone may have resulted from the epinephrine injection and that natural mating then stimulated contractions, resulting in an over-all change greater than that occurring in the control cows. However, before this can be concluded further proof is needed.

The effects of oxytocin injections on the percentage of conceptions in hard-to-settle cows are shown in Table 2. The cow numbers are limited and the differences, though large, are not statistically significant. In some of these cows there

TABLE 2

Effect of injecting oxytocin on conception rate in hard-to-settle cows

	Naturally mated		Artificial insemination		Total	
	Control	Oxytocin	Control	Oxytocin	Control	Oxytocin
No. cows	57	29	14	14	71	43
Cows calving	22	15	3	8	25	23
Per cent conception	39[a]	52[a]	21[b]	58[b]	35	54

[a] Actual calvings.
[b] 60- to 90-day nonreturn.

was a suggestion of a deficiency in release of oxytocin produced by natural mating or artificial insemination.

The authors are well aware of the need for greater numbers of animals in experiments of the type involved here. However, time and the availability of animals have limited the numbers obtained. Although significant differences were not evident in all of the experiments, the trends suggest that oxytocin deficiency may be involved in some cases where cows fail to conceive. The results suggest that this may be more frequent in natural mating than in artificial insemination. However, conception rate in hard-to-settle cows seemed to be improved by oxytocin injections, both in natural mating and artificial insemination.

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SOURCES OF PROGESTINS IN THE PREGNANT COW [1, 2]

J. GORSKI, R. E. ERB, W. M. DICKSON, AND H. C. BUTLER
Departments of Dairy Science, Veterinary Physiology and Pharmacology, and Veterinary Clinical Medicine and Surgery, State College of Washington, Pullman

SUMMARY

Progesterone and Δ^4-pregnene-20β-ol-3-one were assayed, using paper chromatography and ultraviolet spectra. Neither progestin was detected in the placenta or uterine venous blood of a Jersey heifer pregnant 258 days. The blood, adrenals, and testis of the fetus were negative for progestins. The ovaries contained an estimated 50 μg. of progesterone (adrenals < 10 μg.) and 5 μg. of Δ^4-pregnene-20β-ol-3-one. Six additional assays of cow placentae have been negative for progesterone. Corpora lutea (five assays involving three each) contained approximately 90% of the progesterone and all of the Δ^4-pregnene-20β-ol-3-one found in the ovaries of cows. The remaining progesterone was in the ovaries from which the corpora lutea were removed. Progesterone ranged from 6.4 to 15.2 μg/g in luteal tissue. The lowest concentration was from late-pregnant and the highest from nonpregnant cows.

In mammals the corpus luteum is essential for pregnancy for variable periods of time depending on the species involved. The corpus luteum appears essential throughout gestation in the rat *(17)*, goat *(8)*, and rabbit *(13)* and for lesser periods in the sheep *(6)*, mare *(15)*, dog *(7)*, guinea pig *(19)*, cat *(7)*, monkey *(16)*, and human *(28)*. In the cow the corpus luteum is essential for approximately 200 days *(20)*. There is evidence that a longer period is desirable in some animals to avoid early parturition and/or retained placentae *(21)*.

It has been well established that late pregnancy or term placenta of the human *(30)* and mare *(26)* is a potent source of progestins. Adler *et al. (1)* and Ehrhardt and Hardt *(10)*, using bioassay, were able to demonstrate progestational activity in cow-placental extracts. On the other hand, Short *(25)* was unable to detect progesterone chromatographically in extracts of kilogram quantities of cow-placenta late in pregnancy or at parturition. Placentae of the ewe, sow, and bitch likewise were negative *(25)*. Neher and Zarrow *(22)*, using the Hooker-Forbes bioassay, found that levels of progestin in peripheral blood of pregnant ewes increased during pregnancy. They also reported that ovariectomy performed as early as the 60th day of pregnancy had no effect. However, Edgar *(9)*, using chromatographic procedures, was unable to detect progesterone in the peripheral blood of pregnant or nonpregnant sheep or in blood from the uterine vein of a 30-day pregnant ewe. Edgar *(9)* did find an estimated 2 μg/ml of progesterone in the ovarian vein blood of a 98-day and a 3.5-mo. pregnant ewe. Neher and Zarrow *(22)* point out that there may be another progestational hormone having greater biological activity than progesterone, because bioassay has consistently given higher values than chemical procedures.

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Species vary in the length of time the corpus luteum is required for pregnancy maintenance. This fact, plus inability to show that the placenta is a rich source of progesterone during late pregnancy in the cow, ewe, sow, and bitch, has focused attention on sources and identification of other progestins.

Short *(27)* showed an extraovarian source of progesterone in five ewes by ovariectomizing at the 60th day of pregnancy and collecting blood for chromatographic analyses at 100 days. Progesterone and another more polar material, probably Δ4-pregnene-20α-ol-3-one, were observed on the chromatograms of plasma extract from each ewe.

Raeside and Turner *(23)* were unable to detect progesterone in the uterine venous blood of the pregnant goat, though high concentrations were found in ovarian venous blood. Forbes *(11)*, using the Hooker-Forbes bioassay, detected progestions in the umbilical cord blood of goat fetuses. In three out of five, the levels were higher in the umbilical artery than in the umbilical vein. Forbes *(12)* also found progestin levels higher in the embryos than in the placentae of mice.

Progesterone has been isolated from bovine adrenal extracts *(4)* and in the adrenal venous effluent of pregnant cows, bull calves, and pregnant and nonpregnant sheep *(3)*. Edgar *(9)* has isolated progesterone from the corpus luteum of a calf following injection of gonadotrophin and from the corpus luteum and follicular fluid of sheep ovaries.

In addition to progesterone, Δ4-pregnene-20β-ol-3-one has been identified in placenta of the mare *(26)* and human *(31)*, and Δ4-pregnene-20α-ol-3-one has been identified in the latter *(31)*. Zander *et al. (31)* have shown that progesterone and both 20-hydroxy derivations of progesterone increase in the human placenta during pregnancy, whereas levels in the corpus luteum remain relatively unchanged. Wiest *(29)* has examined the blood plasma, ovaries, placentae, fetal membranes, amniotic fluid, and residual uteri of pregnant rats. He detected progesterone and Δ4-pregnene-20α-ol-3-one only in the ovaries and blood plasma.

Gorski *et al. (14)* have identified progesterone and Δ4-pregnene-20β-ol-3-one in ovaries of nonpregnant heifers and cows.

It was the purpose of the study reported here to seek sources of progestins in the pregnant cow.

METHODS

This study was carried out in two parts. In the first, a Jersey heifer, pregnant for 258 days, was anesthetized with Na pentobarbital. An incision was made in the right flank and a catheter was placed in the uterine vein. The animal was given an injection of heparin and four liters of uterine venous blood were collected in serial lots of one liter each. The living male calf was then removed from the uterus and 57 ml. of calf blood were collected by heart puncture. The cow and calf were then sacrificed. The placenta, adrenals, and ovaries from the cow and adrenals and testis from the calf were removed.

In the second part, ovaries were collected at a packing plant immediately

after slaughter. The ovaries were grouped into three approximate reproductive stages: *(a)* Late pregnancy (40–80 cm. fetus—approximately 150–270 days); *(b)* early pregnancy (< 20 cm. fetus—approximately 30–120 days), and *(c)* nonpregnant (control). The ovaries from each cow within the just-described reproductive stages were separated into three groups: *(a)* Corpus luteum of pregnancy or post-estrus in nonpregnant cows; *(b)* remainder of ovary (stroma) after removal of the corpus luteum, and *(c)* the opposite ovary which contained no corpus luteum (Ovary-2).

The ovaries of three animals in similar reproductive stages were placed in each ovary-separation group to increase the quantity of progestins available for each extraction. Two groups of three each were run from the early pregnant and late pregnant stages and one group of three from the nonpregnant stage (control).

All tissues were carried through basically the same procedure. Tissues were extracted with ethyl acetate in a Waring blendor. Blood was extracted with mixtures of benzene and ethyl acetate. The solvents were then removed by vacuum distillation at 45° C. The resulting residue was extracted with warm 70% methanol and the lipids removed by cooling and filtration at −10° C. as suggested by Allen *(2)*. The methanol was evaporated from the filtrate at 45° C. under a stream of nitrogen. The aqueous fraction, made up to 15 ml. by the addition of water, was extracted with ethyl acetate.

After removal of the ethyl acetate, the residue was chromatographed on paper in the Zaffaroni formamide–Skellysolve B system to separate the known progestins *(30)*. Progesterone and △4-pregnene-20β-ol-3-one were detected as areas of absorption by an ultraviolet light scanner. These areas were then eluted and rechromatographed in the Bush 95% methanol–Skellysolve B system *(5)*. Ultraviolet absorbing spots were again eluted and their ultraviolet absorption spectra determined. Quantitative estimates were made by a procedure similar to that outlined by Reineke *(24)*, which was based on the method of Johnson *et al. (18)*. Where quantities were low, visual estimates were made from paper chromatograms, using an ultraviolet light scanner.

As types and quantities of tissue varied a great deal, the quantitative estimates should be considered only on a relative basis. This method allows detection of 10-μg. quantities of progesterone added to 10 g. of placental tissue. Recovery with 10 and 50 μg. of progesterone added to 10 g. of placental tissue was 59%. Short *(27)* recovered 50% of extra progesterone added to ox plasma. Short *(27)* used a modification of the method described by Zander and Simmer *(32)*.

RESULTS

As Table 1 shows, 50 μg. of progesterone (3.7 μg/g of tissue) were extracted from the ovaries of the late pregnant heifer. A detectable amount of △4-pregnene-20β-ol-3-one was also found. Gorski *et al. (14)* had previously extracted an average of 4.3 μg. of progesterone and 0.79 μg of △4-pregnene-20β-ol-3-one per gram of ovaries composited from nonpregnant cows and heifers. Of the remain-

TABLE 1

Progestins in the blood and tissues of a Jersey heifer and fetus sacrificed on the 258th day of pregnancy

Tissue source	Volume or weight of tissue extracted	μg. of progesterone	μg. of Δ4-pregnene-20β-ol-3-one
Ovaries	13.6 g.	50	5[a]
Placenta	1,400 g.	[b]	[b]
Uterine venous blood			
whole blood (1st liter)	1,000 ml.	[b]	[b]
plasma (2nd liter)	305 ml.	[b]	[b]
Cow adrenals	17.9 g.	10[a]	[b]
Calf blood	57.0 ml.	[b]	[b]
Calf adrenals	2.0 g.	[b]	[b]
Calf testis (one testis extracted)	2.2 g.	[b]	[b]

[a] Visual estimate from paper chromatogram.
[b] Not detectable.

ing heifer and calf tissues extracted, progesterone was detected only in the heifer adrenals.

Other assays for progestins in the placentae from six parturient and preparturient cows have all been negative.

Corpora lutea (Table 2) were the major sources of progesterone and Δ4-pregnene-20β-ol-3-one. The quantity of progesterone was three to five times greater. There was a tendency toward higher concentrations of progesterone in the sample of three corpora lutea from nonpregnant cows (15.2 μg/g) as compared with 10.8 and 8.2 μg/g, respectively, for early- and late-pregnant cows. Though a trend was noted, there was considerable sample-to-sample variation. Sample 5 (late pregnancy) averaged slightly higher than Sample 3 (early pregnancy), thus indicating the need for a greater number of assays.

As further shown (Table 2), approximately 10% of the total progesterone extracted from the ovary containing the corpus luteum of pregnancy was from the extraluteal portions. Δ4-Pregnene-20β-ol-3-one, if present at all, was in quantities too low to be detected. Neither progestin was detected in the opposite ovaries which originally had no corpora lutea.

The average for combined ovarian and luteal tissue from the late pregnancy group (Table 2) was 3.2 μg. of progesterone per gram of tissue as similarly compared with 3.7 μg. for the 258-day pregnant Jersey heifer (Table 1).

DISCUSSION

Zander *et al. (31)* have shown that progestin levels remain relatively unchanged in the corpora lutea during pregnancy in humans. The present study indicates that the cow ovary contains relatively similar quantities of progestins during pregnancy and nonpregnancy. Failure to detect progestins in term placentae is in agreement with Short *(25)*. These results suggest *(a)* that the placenta is not a source of extraovarian progestin or *(b)* that an unidentified placental progestin is present. This latter possibility should be considered further because methods employing bioassay have detected progestational activity in

TABLE 2

Distribution of progesterone and Δ4-pregnene-20β-ol-3-one (20β-ol) in the ovaries of the cow

	Corpora lutea					Ovaries minus their corpora lutea					Opposite ovary				
		Progesterone		20β-ol			Progesterone		20β-ol			Progesterone		20β-ol	
Sample	Tissue	Total	av/g	Total	av/g	Tissue	Total	av/g	Total	av/g	Tissue	Total	av/g	Total	av/g
(No.)	*(g.)*	*(μg.)*				*(g.)*	*(μg.)*				*(g.)*	*(μg.)*			
							Nonpregnant								
1	9.9	150.6	15.2	27.6	2.8	20.0	5[a]	0.2	[b]	....	20.0	[b]	....	[b]	....
							Early-pregnant (30–120 days)								
2	10.4	137.0	13.3	29.5	2.8	40.2	11.0	0.3	[b]	....	30.0	[b]	....	[b]	....
3	10.7	91.5	8.6	39.2	3.7	30.3	11.2	0.4	[b]	....	40.0	[b]	....	[b]	....
Av.	10.6	114.2	10.8	34.4	3.2	35.2	11.1	0.3	....	....	35.0	....	....	....	....
							Late pregnancy (150–280 days)								
4	10.7	69.0	6.4	26.8	2.5	13.0	7.7	0.6	[b]	....	8.5	[b]	....	[b]	....
5	12.0	118.5	9.9	16.3	1.4	9.8	10.8	1.1	[b]	....	10.4	[b]	....	[b]	....
Av.	11.4	93.8	8.2	21.6	1.9	11.4	9.2	0.8	....	....	9.4	....	....	....	....

[a] Visual estimate from paper chromatogram.
[b] Not detectable.

blood and tissues from which little or no progestin can be extracted chemically, using present procedures *(22)*.

Extraovarian sources of progestins have been demonstrated for pregnant sheep *(27)*. Progestins have been found in cow-adrenal extracts *(4)*, adrenal venous effluent of bull calves and pregnant cows *(3)*, and umbilical-cord blood of goat fetuses *(11)*.

Raeside and Turner *(23)* were unable to detect progesterone in uterine venous blood from pregnant goats. Wiest *(29)* was unable to extract progestins from placentae, fetal membranes, amniotic fluid, and residual uteri from pregnant rats. These results, in general, agree with those shown in Table 1.

Though ovaries and corpora lutea of several species contain progestins during pregnancy, certain differences can be noted. Zander *et al. (31)* found an average of 5.8 μg. of progesterone per gram of luteal tissue from women in the ninth and tenth months of pregnancy. The late-pregnancy group of cows similarly averaged 8.2 μg/g (Table 2) and late-pregnant rats *(29)* showed a range of 9–12 μg/g of whole ovaries. Zander *et al. (31)* were unable to quantitate, but did detect in two of six cases the 20-hydroxy derivatives of progesterone. The late-pregnant cows averaged 1.9 μg/g of corpus luteum (Table 2) of the 20β derivative. The late-pregnant rats ranged from 8 to 13 μg. of the 20α derivative per gram of whole ovaries. These comparisons are particularly interesting because the human requires a functional corpus luteum during early pregnancy, the cow past mid-pregnancy, and the rat throughout pregnancy.

Recoveries of the progestins may have been lower in the present study than those of Zander *et al. (31)* and Wiest *(29)*, because our extracts were rechromatographed, thereby requiring an extra elution from the paper.

The results of this study suggest that the corpus luteum of dairy cows produces progestins throughout pregnancy and may be the major source of progestins during pregnancy. The fact that some cows fail to abort following enucleation of the corpus luteum after the 200th day of pregnancy does not conclusively show that no progestin is required, because *(a)* some cows calve earlier than expected, *(b)* ovarian tissue and possibly some luteal tissue may remain, *(c)* progesterone is present in adrenal venous blood *(3)*, and *(d)* unidentified progestins may be present.

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EFFECT OF ESTROGEN AND PROGESTERONE ON THYROID FUNCTION OF CATTLE [1,2]

G. W. PIPES, B. N. PREMACHANDRA, AND C. W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

SUMMARY

A preliminary study is presented concerning the possible role of the ovarian hormones (estrogen and progesterone) upon the rate of secretion of thyroid hormone during estrus and pregnancy. For this purpose, a technique was developed consisting of the measurement of thyroidal-I^{131} release when recycling of I^{131} was prevented by a goitrogen (thiouracil) during a preliminary control period. During the subsequent period of ovarian hormone injection, the measurement of release rate is continued. If a change in release rate is noted, it is believed to indicate that a change in thyroxine secretion rate has been induced. The injection of 100 mg. per day of progesterone alone or in combination with 100 μg. of estradiol benzoate failed to alter the release rate of thyroidal I^{131} in groups of dairy cattle. While 1.15 mg. estradiol benzoate alone also failed, when 2.5 or 3.0 mg. were injected daily, the release rate was increased to a statistically significant extent. These observations are interpreted as indicating that the level of estrogen secreted in late pregnancy stimulates increased secretion of thyrotropin and thyroxine during early lactation.

One of the basic patterns of control of thyroid gland function resides in the action of the pituitary thyrotropic hormone upon the thyroid gland and the action of the thyroid hormone upon the pituitary. Thus, under constant conditions of environment (nutrition and temperature), the secretion of thyroxine would tend to be constant. Under these conditions, the variation in thyroxine secretion rate in animals might be ascribed to genetic differences. In a previous paper *(12)*, a study was reported concerning genetic differences and seasonal changes (ambient temperature) upon thyroxine secretion rate of cattle under uniform nutritional conditions.

The present paper is concerned with the possible influence of the internal environment of the dairy cow during reproduction upon thyroxine secretion rate. This would include changes which might occur during estrous cycles, pregnancy, and lactation. Since the determination of thyroxine secretion rate requires a certain amount of time (about 2 wk. or more), short-time changes are difficult to measure. In beginning this study, it seemed of interest first to explore the influence of the two ovarian hormones separately and together, to note their effect upon thyroid function, before attempting to evaluate the more complicated normal physiological changes during estrus and pregnancy.

In experimental animals, many studies have been reported concerning the influence of estrogen upon thyroid function. In these studies, many indices of thyroid function have been employed, such as I^{131} uptake, thyroidal-I^{131} release rates, thyroid cytology, and hyperplasia. Estrogen has been reported to increase

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thyroid activity, as shown by I^{131} uptake of the thyroid gland *(5, 7, 8, 9, 17)*, by the thiouracil-thyroxine secretion rate *(6)*, by increase in the PBI of blood *(4)*, and by increased hypertrophy of the thyroid gland *(3)*. It is of interest to note that Clifton and Meyer *(3)* found that in rats estrogen depressed appetite and hypertrophy of the thyroid, but that force-fed, estrogen-treated rats showed thyroid hypertrophy. However, Noach *(9)* found increased I^{131} uptake during estrogen administration when iodine intake was controlled. Under similar conditions, Soliman and Reineke *(15)* found increased uptake during estrus in the rat. This finding had been previously demonstrated in the rodent by I^{131} uptake *(14, 16)* under uncontrolled iodine intake.

Large doses of estrogen have been reported to have no effect on I^{131} uptake *(7, 10)* in the rat and to inhibit the release of thyroidal I^{131} in the rabbit *(1)*.

Spaying has been found to produce no change in I^{131} uptake in the rat *(10)* and to produce no change in release rate of thyroidal I^{131} in the rabbit *(2)*.

Progesterone *(7, 8)* has been reported to increase I^{131} uptake in the rat, but when injected concurrently with estrogen *(14, 17)* it has been shown to decrease uptake.

From the review of the literature, it is difficult to determine the role of the ovarian hormones, administered in physiological amounts, in the regulation of thyroid function during reproduction. Further, studies of this problem have not been conducted previously with domestic animals such as cattle.

Indices of thyroid function. Since I^{131} uptake by the thyroid gland appears to be subject to many influences, its value as an index of thyroid function appears to be limited. The release rate of thyroidal-I^{131} when recycling of iodine is prevented by the use of goitrogens appears to be a more useful measure of thyroid function *(11)*. It is not as satisfactory as thyroxine secretion rate determination, for reasons pointed out by Premachandra *et al. (12)*. However, by the use of the same animals, in which the rate of release of thyroidal-I^{131} is first determined (control period), followed immediately by an experimental period during which the hormone is administered, changes in the release rate may be considered to indicate altered thyroid function.

In using the thyroxine secretion rate technique, the control and experimental periods would require the elapse of about 1 mo. Since the ambient temperature of our cattle can not be controlled, temperature changes might influence the results during these longer time intervals. As a preliminary to such studies, it seemed of value to determine the possible influence of the ovarian hormones on the thyroidal-I^{131} release rate in cattle.

EXPERIMENTAL PROCEDURE

Cows and calves in the experimental herd were injected intravenously with 200–300 μc. of carrier-free radioiodine (NaI^{131}). Daily measurements of thyroidal-I^{131} were made by the method described by Pipes *et al. (11)*. After maximum accumulation of I^{131} was observed in the thyroid, the release phase was measured for a number of days. The rate of decline thus observed served as a control period for the normal rate of release of thyroidal-I^{131} in the individual

animals. In most of the studies, thiouracil was administered in sufficient amounts to prevent the reutilization by the thyroid of I^{131} from metabolized thyroid hormone. Thiouracil was administered daily by gelatin capsule in 12- or 24-g. doses.

Estradiol benzoate and progesterone were dissolved in olive oil and injected subcutaneously during the test periods. The animals were allowed access to iodized salt blocks at all times.

Conventional corrections were made for background and for physical decay of the isotope. Results were expressed as per cent of injected dose.

Release rates of thyroidal I^{131} were calculated according to the methods previously described *(11)* with the term k″4 being employed for release rate constant per hour during thiouracil administration. For comparison, the daily percentage release rate is presented. These values indicate the percentage of the total I^{131} present in the thyroid glands which is released each 24 hr.

RESULTS

Estrogen and progesterone. During the first two-thirds of pregnancy, estrogen and progesterone are secreted to stimulate growth of the udder. While the levels of these two hormones normally secreted at this time are not known, Turner *et al. (19)* reported that the daily injection of 100 μg. estradiol benzoate and 100 mg. progesterone for 180 days was believed to stimulate satisfactory udder growth in heifers *(13)*.

It was of interest, therefore, to determine the possible influence of these two hormones of pregnancy upon thyroid function, uncomplicated by fetal growth. Thiouracil was administered to five dairy heifers and two bull calves during a six-day control period, to determine the normal rate of release of thyroidal-I^{131}. The ovarian hormones at the above levels were then injected daily for a period of eight days. If these hormones stimulated increased secretion of thyrotropin, it should be reflected by an increased rate of release of thyroidal-I^{131}. It will be seen that no change was observed in the release rate during the injection of 100 μg. estradiol benzoate and 100 mg. progesterone (Table 1). Typical data on

TABLE 1

Effect of estrogen and progesterone on thyriodal-I^{131} release with concurrent administration of thiouracil

Experiment (daily injection)	No. of Animals	Release rates per hour (k″ 4) under thiouracil administration	
		Control	Experimental
Progesterone, 100 mg. Estrogen, 100 μg.	7	0.0042±0.0006 (10.08%)[a]	0.0044±0.0007 (10.56%)
Estradiol benzoate 1.15 mg.	9	0.0024±0.0004 (5.76%)	0.0018±0.0003 (4.32%)
Estradiol benzoate 2.5 mg.	2	0.0041±0.0001 (9.84%)	0.0060±0.0011[b] (14.40%)
Estradiol benzoate 3 mg.	7	0.0055±0.0001 (13.20%)	0.0073±0.002[b] (17.52%)

[a] Each value in parentheses indicates the percentage of I^{131} present in the thyroid glands which is released each 24 hr. = k″4 × 24 ×100.

[b] Increase statistically significant ($P < .01$).

two heifers (No. 89 and 76) and one bull calf (H1) is presented (Figure 1). These data suggest that the ovarian hormones secreted during the udder growth phase of pregnancy do not stimulate increased thyroxine secretion.

Progesterone. To determine whether this level of progesterone alone would stimulate increased thyrotropin secretion, twin heifers were run during a control period of six days, then injected with progesterone (100 mg. daily) for seven days. Since the release rate of thyroidal-I^{131} was unchanged during the period

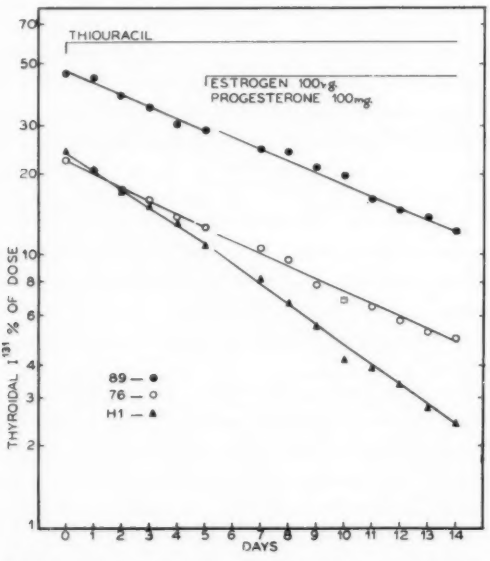

FIG. 1. The injection of a combination of estrogen and progesterone comparable to that secreted during early pregnancy beginning on the seventh day is shown not to change the rate of release of thyroidal-I^{131} from that of the first six-day control period (see also Table 1).

of hormone injection, it was concluded that neither estrogen and progesterone nor progesterone alone at these levels influenced pituitary-thyroid relations (Figure 2).

Estrogen. In late pregnancy, the secretion of estrogen by the cow increases markedly. It is well known that estrogen, at this time, stimulates the increased proliferation of the acidophilic cells and secretion of the lactogenic hormone. For this reason, Turner *et al. (19)* administered 3 mg/day of estradiol benzoate to initiate lactation in heifers whose mammary glands had been grown previously with estrogen and progesterone. The question arose whether the estrogen level in late pregnancy might stimulate an increased secretion of thyrotropin and thyroxine and thus give the milk-secretion process an added boost.

In a series of experiments, increasing amounts of estrogen were given to

heifers and bull calves and the rates of release of thyroidal-I^{131} observed after a preliminary control period. When 1.15 mg/day of estradiol benzoate was administered to seven heifers and two bull calves, no change in release rate was observed (Table 1). However, when the level of estrogen was increased to 2.5 mg. daily, the release rate of I^{131} was distinctly increased in two bull calves in comparison to the control period. At the 3.0-mg. level (which stimulates the initiation of milk secretion) two bull calves and five dairy heifers showed increases in

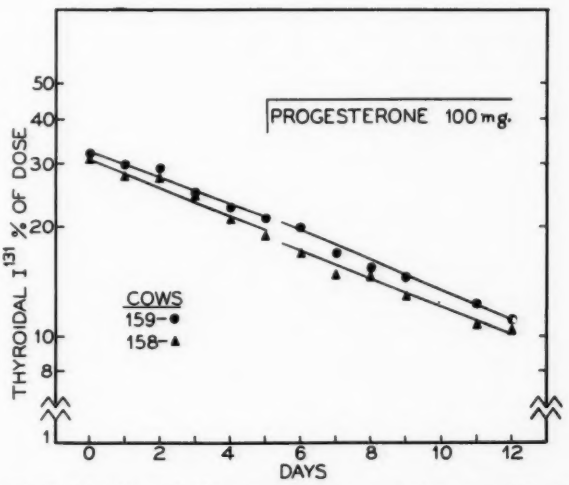

FIG. 2. The daily injection of 100 mg. progesterone during a period of seven days is shown not to change the rate of release of thyroidal-I^{131} from that observed during the first six-day control period.

thyroidal-I^{131} release rates which were statistically significant when compared to their control periods (Table 1). Instead of the continued normal release rate, a rapid increase in release rate was observed (Figure 3).

The increased release rate of thyroidal-I^{131} in itself indicates an increased daily rate of discharge of thyroxine into the blood and concurrently an increased rate of secretion of thyroxine. Since thyroid gland function is controlled by the thyrotropic hormone of the anterior pituitary, it is inferred that estrogen acts upon the pituitary to increase the rate of secretion of thyrotropin.

The role of increasing estrogen production in late pregnancy, in stimulating the increasing secretion of the lactogenic hormone of the pituitary and the initiation of milk secretion at parturition, has been recognized for some time. The present observations indicate a second important function of estrogen at this time; namely, to stimulate increased secretion of thyrotropin and thyroxine, to increase the intensity of milk secretion, and to promote a rapid rise in milk secretion postpartum.

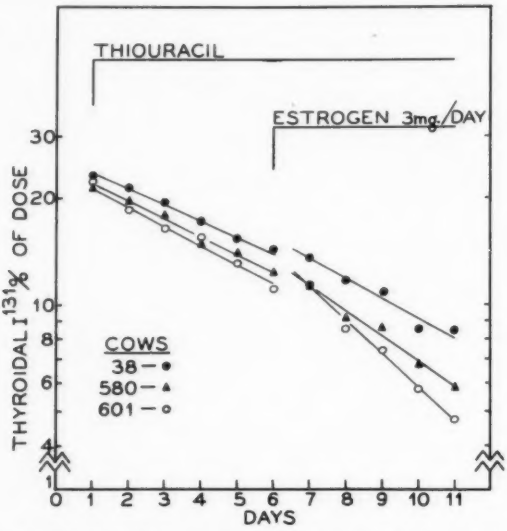

FIG. 3. The daily injection of 3 mg. estradiol benzoate starting on the seventh day increased the rate of release of thyroidal-I^{131}, compared to the release rate during the preceding six-day control periods. This change is interpreted as indicating that estrogen at the physiological level stimulates the increased secretion of thyrotropin and thyroxine.

DISCUSSION

The endocrine interaction which stimulates the growth of the mammary glands of the udder of cattle during the first two-thirds of pregnancy, and later stimulates the initiation of milk secretion, is of great importance in understanding the fundamental causes of variation in the milk production of dairy cattle. Do the ovarian hormones (estrogen and progesterone) which play vital roles in stimulating mammary gland growth and the initiation of lactation interact with other endocrine glands to make possible the marked rise in milk secretion which occurs postpartum?

The data presented in this paper suggest that estrogen and progesterone in amounts and ratios which stimulate mammary gland growth have little influence upon thyrotropin secretion. However, the increasing level of estrogen secreted in late pregnancy not only stimulates an increased secretion of lactogenic hormone by the pituitary but also may stimulate the secretion of increased amounts of thyrotropin and correspondingly more thyroxine. Since it is well known that thyroxine stimulates more intense milk secretion, this effect could be expected to aid cows in reaching maximum levels of milk production.

Since estrogen secretion is discontinued at parturition, the stimulus to increased thyrotropin secretion would gradually decline. This might explain why the feeding of thyroprotein to dairy cattle is of less value during the rising segment and at the peak of production than when lactation begins to decline.

Feeding thyroprotein during normal declining lactation would thus maintain thyroxine levels of the blood, comparable to or above that initially stimulated by estrogen in late pregnancy.

Finally, it should be pointed out that estrogen apparently influences the secretion of pituitary growth hormone. This is the explanation of the favorable influence of diethylstilbestrol upon the growth of beef cattle. It has been shown by Struempler and Burroughs *(18)* that either diethylstilbestrol or growth hormone resulted in increased nitrogen retention in lambs. They concluded that diethylstilbestrol increases nitrogen retention in ruminants through the increased release of growth hormone in the animal's body.

From these varied observations, it is suggested that the secretion of high levels of estrogen in late pregnancy stimulates the secretion of increased levels of three anterior pituitary hormones, lactogen, growth, and thyrotropin, all of which influence the intensity of milk secretion.

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GLYCOLYTIC ACTIVITY IN MIXED SEMEN SAMPLES [1,2]

M. H. EHLERS, N. S. KUSHWAHA, AND R. E. ERB
Department of Dairy Science, State College of Washington, Pullman

SUMMARY

Glycolytic activity in mixed semen was compared by measuring fructose and lactic acid changes during 1 hr. of incubation at 37° C. A mix was made of the semen from two bulls and ultimately semen from each bull was mixed with semen from each of the other six bulls. Mixing of semen from two bulls at a time was replicated four times for a total of 84 mixes with accompanying unmixed controls.

On an over-all basis, fructose utilization and lactic acid accumulation were stimulated by mixing; lactic acid changes were somewhat less pronounced. Mean values showed mixed sample fructose utilization to be 8% higher. A part of this increase appeared to be due to a more favorable fructose supply in some samples owing to mixing, but other stimulation related to mixing of semen from two sources is probably involved. Although semen from the different bulls varied substantially in quality, there was no strong individual bull influence contributing to the stimulation.

Hess *et al. (9)* reported that mixing semen of three or four bulls gave a higher nonreturn rate than unmixed control semen. The mixed semen had a greater percentage of live sperm and better motility during seven days of refrigerated storage. Campbell and Jaffe *(3)*, however, observed no improvement in motility with mixing. Gassner *et al. (7)* found that mixing of bull semen had no detrimental effects on the ability of sperm to use fructose. Mixing of semen obviously can have little direct application in cattle breeding. However, it may be possible to use this information, if the nature of any beneficial effects can be established. Enhancement of fertility would appear to be due to having sperm from more than one source, to having seminal plasma from more than one source, or to both.

The purpose of this experiment was to determine whether mixing affected glycolytic activity of semen. Three principal alternative results were visualized: *(a)* no change in glycolysis; *(b)* stimulation; *(c)* inhibition. None of these excluded the others, because semen samples might vary in response to mixing. Beneficial effects might not necessarily be those associated with stimulation; it is conceivable that inhibition of glycolysis is desirable.

EXPERIMENTAL PLANS AND PROCEDURES

Seven bulls producing semen with substantial variation in activity were selected on the basis of pre-experimental information. With such semen, any effects due to mixing should be more detectable. These bulls were from the Angus, Guernsey, Holstein, and Jersey breeds, and ranged from 2 to 7 yr. in age.

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Semen from each bull was mixed with semen from each of the other six bulls. A given mixing was made with semen from two bulls. Thus, there were 21 combinations of mixed semen from the seven animals. Combinations were replicated four times for a total of 84 mixings. Each mix had as its controls the unmixed semen from the two contributing samples. About 15 min. elapsed between collection and the beginning of controlled incubation. The mixed and the unmixed portions were incubated under essentially anaerobic conditions with four parts of citrate buffer for 60 min. at 37° C. The general procedure has been previously described *(6)*. Fructose measurement was by the method of Roe *(13)* as modified by this laboratory *(6)*. Lactic acid determination was as outlined by Barker and Summerson *(1)*, except that 0.5 g. of calcium hydroxide was used. For the initial and 60-min. lactic acid measurements, filtrate equivalent was 0.05 and 0.01 ml. of semen, respectively.

Fructose and lactic acid changes during 60 min. of incubation were expressed on a "per milliliter of semen" and "10^9 sperm" basis. Estimated fructose changes in 10 min. were derived using first-order reaction formulae *(6, 10)*. "Expected" fructose and lactic acid changes per 1 ml. mixed semen = actual change in ½ ml. of semen from one contributing bull (first control) + actual change in ½ ml. of semen from the other contributor (second control). Expected changes by 10^9 sperm were calculated from these values.

Differences between the expected glycolytic changes and those actually observed in mixed semen were examined statistically by the "t" test *(14)*. Motility estimates were on the basis of percentage progressively motile at the beginning of incubation in the citrate-diluted semen. Motility was not rated in mixed semen because of the subjective nature of such evaluation. Sperm concentration was determined by hemocytometer.

RESULTS

Table 1 shows glycolytic data for the unmixed controls to serve as a comparison with other studies. The desired variability of semen was achieved as indicated in the fructose utilization by 10^9 sperm in 60 min., where bull means ranged from 1.24 to 2.31 mg. Motility ranged from 45 to 69%, and other values in Table 1 show sizable differences between bulls. Note, however, that none of the bulls produced semen having extremely high levels of fructose *(6)*.

The differences between actual and expected glycolytic changes in mixed semen are shown (Table 2).

Fructose utilization per milliliter of mixed semen averaged 2.00 mg. in 60 min. (Table 2), which was 0.16 mg. higher than expected ($P < 0.01$). Utilization by 10^9 sperm was 2.02 mg., which was higher than expected by 0.18 mg. ($P < 0.01$).

Fructolytic rates depend upon sperm numbers, the amount of fructose, and the length of incubation *(10, 12)*. Therefore, mixing of semen should, with some samples, result in a more favorable sperm-to-fructose ratio, particularly with longer incubations. This would simply be a matter of nutrient availability. Fructolytic rates are generally underestimated in semen that has high numbers of

TABLE 1

Glycolytic data from independently incubated semen
(12 ejaculates per bull)

Bull	Initial motility	Concentration per ml. of semen	Initial fructose per ml. of semen	Fructose utilization				Initial lactic acid per ml. of semen	Lactic acid accumulation	
				Per ml. of semen in 60 min.	By 10^9 sperm in 60 min.	Per ml. of semen in 10 min.[a]	By 10^9 sperm in 10 min.[a]		Per ml. of semen in 60 min.	By 10^9 sperm in 60 min.
	(%)	*(10^9 sperm)*	*(mg.)*							
G–9	62	1.34	5.90	2.18	1.62	0.50	0.36	0.52	1.69	1.21
G–10	52	1.08	3.90	1.34	1.24	0.33	0.31	0.53	1.15	1.07
J–18	69	1.10	4.60	2.05	1.90	0.48	0.43	0.53	1.57	1.51
H–32	69	1.16	5.65	2.54	2.31	0.60	0.53	0.48	1.40	1.25
H–34	69	0.97	5.44	2.02	2.09	0.44	0.44	0.58	1.46	1.54
A–60	45	0.83	3.36	1.22	1.93	0.27	0.39	0.28	0.78	0.95
A–64	57	0.75	3.40	1.53	2.09	0.34	0.46	0.32	1.09	1.40
Mean	60	1.03	4.61	1.84	1.88	0.42	0.41	0.46	1.30	1.28

[a] Estimated on basis of first-order reaction (see Reference *6*).

TABLE 2

Differences between actual and expected glycolytic values for mixed semen
(21 bull combinations; 84 mixes)

Semen from 6 other bulls mixed with:	Measurement in mixed semen	Fructose utilization				Lactic acid accumulation	
		Per ml. semen in 60 min.	By 10^9 sperm in 60 min.	Per ml. semen in 10 min.[a]	By 10^9 sperm in 10 min.[a]	Per ml. semen in 60 min.	By 10^9 sperm in 60 min.
		(mg.)					
G–9	Actual	2.11	1.84	0.47	0.40	1.59	1.35
	Difference[b]	+0.15	+0.18	+0.01	+0.03	+0.18	+0.15
G–10	Actual	1.70	1.51	0.39	0.35	1.27	1.14
	Difference	+0.09	+0.07	0.00	+0.01	−0.05	−0.06
J–18	Actual	1.92	2.00	0.43	0.43	1.40	1.46
	Difference	+0.16	+0.14	+0.02	+0.01	+0.04	+0.03
H–32	Actual	2.47	2.32	0.56	0.52	1.36	1.22
	Difference	+0.18	+0.19	+0.01	+0.02	+0.14	+0.11
H–34	Actual	2.04	2.14	0.44	0.45	1.40	1.42
	Difference	+0.18	+0.24	+0.03	+0.04	+0.00	−0.02
A–60	Actual	1.79	2.11	0.40	0.46	1.09	1.24
	Difference	+0.24	+0.31	+0.06	+0.07	+0.02	+0.04
A–64	Actual	1.95	2.22	0.45	0.51	1.40	1.53
	Difference	+0.11	+0.17	+0.02	+0.04	+0.06	+0.06
All combinations	Actual	2.00	2.02	0.45	0.44	1.36	1.34
	Difference	+0.16	+0.18	+0.03	+0.03	+0.06	+0.04

[a] Estimated on basis of first-order reaction (see Reference *6*).
[b] Difference from expected value based on independent incubation of contributing samples.

sperm or low levels of fructose, or both. Underestimation of fructolysis is accentuated as the incubation time increases. Estimation of fructose utilized in 10 min., using first-order reaction formulae, provides a useful technique in minimizing variation attributable to sperm numbers and amounts of fructose *(6)*. Application of this technique should indicate whether there is stimulation (or perhaps inhibition) of fructolysis apart from that caused by altering sperm and fructose relationships.

Estimates for 10 min. show mixed semen utilization of 0.45 and 0.44 mg. per milliliter semen and by 10^9 sperm, respectively. These exceeded expected values for 10 min. by 0.02 and 0.03 mg. ($P < 0.025$). Mixed semen fructose utilization per milliter semen in 60 min. was 8.0% higher than expected, whereas estimated utilization in 10 min. was 5.1% higher than expected. Comparable utilizations by 10^9 sperm were 9.2 and 7.0% higher than expected. Thus, part of the higher fructose utilization in 60 min. in mixed semen is due to more available substrate, but there also appears to be a fructolytic stimulus from another factor or factors.

On an over-all basis, lactic acid accumulation per milliliter semen in 60 min. and by 10^9 sperm (Table 2) was only slightly higher than expected ($P < 0.20$). There is no present basis for estimating lactic acid changes in a shorter time interval, such as was applied to the fructose values.

The data were also analyzed for individual bull influences on glycolytic changes in mixed semen. Individually, 59 mixes showed more and 25 showed less than expected, or no difference in fructose utilization during 60 min. Of the 84 mixes, however, only 46 showed more lactic acid accumulation than expected.

This further indicates that lactic acid production is less influenced by mixing. Some bull combinations (four mixes to a two-bull combination) had less fructose utilization than the unmixed controls, but when the six combinations to which a bull contributed were considered, actual fructose utilization in mixed samples averaged higher than expected for all bulls (Table 2). With regard to lactic acid accumulation, two of the seven bulls, considering all of their combinations, had less than expected lactic acid accumulation in mixed semen.

The difference between actual and expected fructose utilization per milliliter of semen in 60 min. ranged from + 0.09 mg. for combinations including semen of G-10 to + 0.24 mg. for combinations including A-60 (Table 2). Fiducial limits at the 95% confidence level were calculated for differences between actual and expected fructolytic activity in mixed samples of the individual bulls (as shown in Table 2). At least one of the fiducial limits for the combinations of a particular bull fell within the range established for each of the other bulls. This overlapping of fiducial limits for differences in activity between actual mixed and expected value was true for all of the other measurements of fructose and lactic acid changes. These bulls produced semen having substantial variation in metabolic activity (Table 1), but the differences attributable to mixing did not have a strong individual bull influence.

DISCUSSION

The report of Hess *et al. (9)* indicates that mixing of semen may improve fertility. The present work shows that mixing may increase fructose utilization and to a lesser extent lactic acid accumulation during 60 min. of incubation at 37° C. Insofar as fructolytic aspects are concerned, some of this increase is apparently a result of more available fructose attributable to mixing and particularly evident with longer incubation. However, part of this increase is probably due to other stimulation. Chang *(4)* found that dead sperm from a bull improved the fertilizing capacity of rabbit semen. Thus, there is the possibility that improved fertility and higher glycolytic activity of mixed bull semen are a result of having sperm from more than one source. The other principal alternative explanation for a stimulus is the presence of a foreign seminal plasma. Lardy and Ghosh *(11)* noted no fertility difference between epididymal and ejaculated sperm from several bulls which, in effect, minimizes the contribution of the fluid portion of semen. Change *(4)*, however, found that seminal plasma from the rabbit improved the fertilizing capacity of ejaculated rabbit sperm. Ghosh and Lardy *(8)* reported that seminal fluid of the bull apparently activated sperm through a regulator associated with the epididymal sperm. Bishop and Salisbury *(2)* suggest that some constituent of semen depresses oxygen uptake of whole semen. None of these observations, however, provides a direct answer to the nature of any stimulus attributable to mixing of semen.

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EFFECT OF AGE AND OF DIETARY REGIME ON HEMOGLOBIN AND REDUCING-SUGAR LEVELS IN THE BLOOD OF DAIRY CALVES[1, 2]

L. RATCLIFF, N. L. JACOBSON, AND R. S. ALLEN
Departments of Animal Husbandry and Chemistry, Iowa Agricultural Experiment Station, Ames

SUMMARY

Twelve male and 12 female Holstein calves were fed limited whole milk for 6 wk. and liquid milk replacer for from 3 to 12 wk. by nipple pail. Half the calves of each sex received a milk replacer containing dried skimmilk, dextrose, distillers dried solubles, and soybean oil meal; the others received a similar replacer minus the distillers solubles. Hay and starter were fed to females for 12 wk. and to males after 7 wk. The males were on screens (no bedding) for the first 7 wk. Hemoglobin levels in the females declined to 5 wk. and increased thereafter; values for the males declined for 9 wk. Hemoglobin values for calves fed distillers dried solubles were significantly higher than those for the other calves. Whole blood reducing–sugar values declined for 8 wk., and limiting some of the calves (males) to a liquid diet fed by nipple did not alter appreciably the nature of these trends. Most of the reducing sugar was in the plasma, where trends were similar to those of whole blood. Packed-cell volumes were relatively constant, but reducing-sugar content of the cells decreased steadily and approached zero at 8 wk.

A number of studies in recent years have demonstrated that the reducing-sugar level is much higher in the blood of the calf than in the blood of the older bovine. The reason for this difference, however, has not been clear. McCandless and Dye *(6)* reported that reducing-sugar values decreased with increasing age of calf to about 7 wk. and remained relatively constant thereafter. These workers attributed the decline to development of rumen function. Other workers *(5, 8, 16)* have observed a similar decline, but the change did not appear to be dependent upon rumen development. McCarthy and Kesler *(7)* found a decline in reducing sugar and an increase in fatty acid levels in the blood with increasing age of calf. Goodwin *(3)* also observed the decrease in blood sugar and reported further that the glucose content of the blood cells of the calf is much higher than that in the blood cells of the adult. This decrease was attributed to a difference in the types of blood cells in the calf and the adult. Further investigation of factors related to blood reducing–sugar levels seemed desirable. In view of the changes in hemoglobin levels which occur in the early postnatal life of the calf and the factors related thereto *(12)*, it seemed appropriate to direct attention to this blood component also. Therefore, the present study was designed to evaluate the effects of age and of certain dietary regimes on hemoglobin and reducing-sugar levels in the blood of the calf.

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EXPERIMENTAL PROCEDURE

Twenty-four purebred Holstein calves (12 males and 12 females) were allowed to remain with their dams for three days following birth and subsequently were allotted as shown in Table 1. The only difference between groups was that

TABLE 1
Experimental design

Group	No. of calves	Weeks fed (inclusive)			
		Whole milk	Milk replacer	Starter[a]	Hay[a]
I (No DDS)[b]					
Males	6	1–6	4–12	8–12	8–12
Females	6	1–6	4–12	1–12	1–12
II (DDS)[b]					
Males	6	1–6	4–12	8–12	8–12
Females	6	1–6	4–12	1–12	1–12

[a] Fed ad lib.
[b] DDS = Corn distillers dried solubles.

the milk replacer fed to Group II contained corn distillers dried solubles, whereas that fed to Group I did not. The males in both Groups I and II were kept on screens (no bedding) the first 7 wk., during which time all of their feed was in liquid form from a nipple pail. The calves were fed milk and/or replacer twice daily; the feeding schedules are shown in Table 2 and the composition of the replacers is shown in Table 3. All calves received dry starter, according to the

TABLE 2
Milk and milk replacer feeding schedules

Weeks	Whole milk[a] *(% body wt/day)*	Milk replacer *(% body wt/day)*	
		Dry mix.	Water
1	8	.....	.....
2	9	.....	.....
3	10	.....	.....
4	9	0.2	0.8
5	7	0.6	2.4
6	6–0	0.8–2.0	3.2–8.0
7–12	None	2.0	8.0

[a] Approximately 3.4% fat.

TABLE 3
Composition of milk replacers

Ingredients	Replacer	
	1	2
Dried skimmilk	25 lb.	20 lb.
Dextrose	25 lb.	20 lb.
Corn distillers dried solubles	....	20 lb.
Soybean oil meal	50 lb.	40 lb.
Vitamin A supplement *(5,000 I.U/g)*	50 g.	50 g.
Irradiated yeast *(4,000,000 I.U. vitamin D/lb)*	5.4 g.	5.4 g.

experimental design of Table 1, which was composed, in percentages, of ground yellow corn 25, ground oats 22, soybean oil meal 25, wheat bran 15, cane molasses 10, dicalcium phosphate 2, and salt 1.

Blood samples were drawn from the jugular vein at weekly intervals approximately 8 hr. after the morning feeding of milk and/or replacer. The blood-collection flasks contained potassium oxalate and sodium fluoride, the latter serving to prevent glycolysis. The plasma per cent was determined within 45 min. after the blood samples were drawn. Hemoglobin values were measured by the alkaline hematin technique *(13)*. The Somogyi-Nelson method *(9, 11)* was used in determining reducing-sugar levels both in whole blood and in blood plasma, and the reducing-sugar levels of the blood cells were calculated.

RESULTS AND DISCUSSION

During the first 6 wk., there were no appreciable differences among groups in hemoglobin values and in plasma per cents (Figure 1). Subsequently, the hemoglobin values for the females began to increase, with the group receiving distillers dried solubles showing consistently higher values. The values for the males did not start increasing until the 9th wk., and the group receiving distillers dried solubles increased more than the group without the dried solubles.

Analysis of variance revealed that the average blood hemoglobin values of calves receiving distillers dried solubles were singificantly higher ($P < .05$) at 12 wk. of age than those for calves receiving no dried solubles. Although the

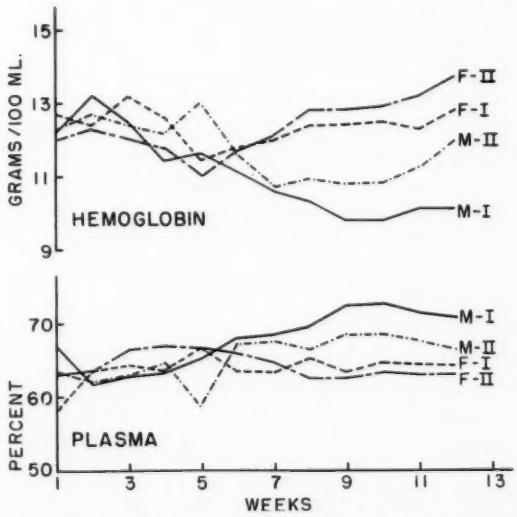

FIG. 1. Effect of age and dietary regime on hemoglobin values and plasma per cent in the blood (M = males, fed only liquids to 7 wk. and liquid, hay, and dry concentrates thereafter; F = females, fed liquid, hay, and concentrates throughout; I = milk replacer contained no corn distillers dried solubles, II = milk replacer contained corn distillers dried solubles.)

hemoglobin values for calves in Group I were within the normal range, the data of this study suggest that distillers dried solubles contain a substance or substances that enhance hemoglobin formation in the dairy calf. The fact that the females were fed hay and starter earlier probably accounts for the higher hemoglobin values for the females. Thomas *et al. (12)* observed that Holstein females had significantly higher blood hemoglobin levels from birth to 75 days of age than did males of this breed. However, in the present study no differences in hemoglobin levels were found between males and females during the first 6 wk. of life. Since the differences reported herein between females and males after 6 wk. of age are much greater than those observed by Thomas and associates, it is unlikely that these differences are due to sex alone.

Thomas *et al. (12)* also found that low hemoglobin levels were associated with subnormal growth. In the present study, however, a relationship between growth and hemoglobin level appears unlikely, since the hemoglobin values were relatively high and all would be considered to be in the normal range. Mean weight gains for the two groups of males were similar, but the females receiving distillers dried solubles showed somewhat greater weight gains than those in Group I. Feed consumption was slightly higher for the animals in Group II.

In general, after 6 wk. the trends for per cent plasma were opposite to those for hemoglobin, a relationship which might be anticipated. A much greater incidence of digestive disturbances was observed among the calves which received no distillers solubles. These disturbances and details of growth responses were discussed elsewhere *(10)*. Although a high incidence of scouring occurred in the calves receiving Replacer I, the per cent plasma was higher and the hemoglobin lower for these calves. The reverse might be expected, since dehydration usually occurs during severe diarrhea. The reason for this phenomenon is not apparent and the need for further research is suggested.

Figure 2 shows the effect of age and dietary regime on the reducing-sugar levels in the plasma and in the cells. The sum of the two values represents the reducing-sugar level in whole blood. The reducing-sugar levels for all groups declined for about 8 wk., an observation which agrees with reports by other workers *(3, 5-8, 16)*. Goodwin *(3)* has proposed, as an explanation for the decrease in corpuscle-reducing sugar, that the red cells in the blood of the young calf are of two main types and that the "foetal" type cell, which is high in reducing sugar, is progressively replaced by the "adult" type cell, which has virtually no reducing sugar. This explanation, however, does not account adequately for the decrease in plasma-reducing sugar. The mean blood reducing-sugar levels for the males declined at a relatively constant rate, the decline averaging 8 mg. per week to 8 wk. of age. The mean weekly decline to 8 wk. was the same for the females, but the rate was more erratic (greater than the values for the males the 1st wk., less from 2 to 6 wk., greater from 6 to 7, and less from 7 to 8). It is possible that sex may influence to some degree the postnatal changes in blood reducing-sugar levels. Voelker *et al. (14)* found that values for males were slightly higher than for females. In the present experiment, effect of sex could not be determined, because of ration differences.

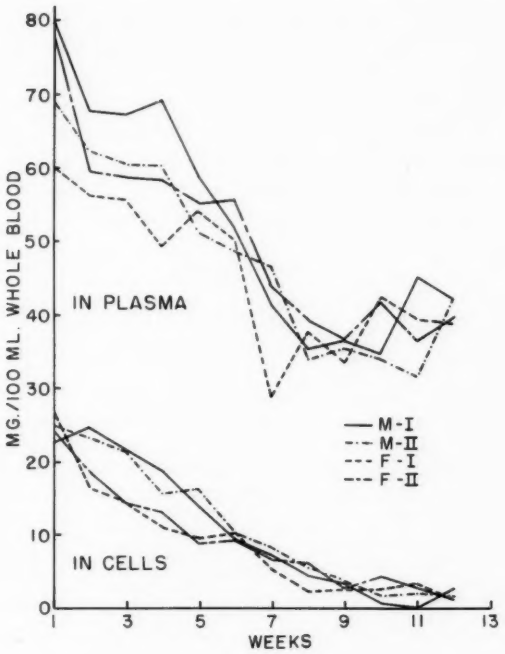

FIG. 2. Effect of age and dietary regime on reducing-sugar levels in blood plasma and cells (M = males, fed only liquids to 7 wk. and liquid, hay, and dry concentrates thereafter; F = females, fed liquid, hay, and concentrates throughout; I = milk replacer contained no corn distillers dried solubles, II = milk replacer contained corn distillers dried solubles).

Warner *et al. (15)* have shown that there is little, if any, rumen activity and development in calves fed an all-milk diet. The males in the present experiment consumed only milk for 3 wk. and only liquid feed by nipple for 7 wk. Thus, most of the feed probably passed to the omaso-abomasal area. Moreover, the construction of the pens was such that no bedding was used. Therefore, little if any rumen development was probable. It is doubtful, then, that rumen development was the cause for the early decline in plasma-reducing sugar of the males in the present study. The results reported by Lambert *et al. (5)*, Murley *et al. (8)*, and Wing *et al. (16)* support this reasoning. However, Hibbs and coworkers *(4)* observed higher blood glucose values in calves continued on whole milk beyond 7 wk. of age than in calves of the same age which were fed only hay and dry concentrates.

There probably are several factors, working together in the metabolic systems of the ruminant, that are responsible for this change from a high to a low blood-sugar level. Since a decrease occurs, regardless of dietary regime and rumen development, it is very probable that the metabolic mechanisms change first, and that the rumen normally develops at a rate sufficient to supply the necessary

metabolites (perhaps the short-chain fatty acids) to meet the demands of these mechanisms. This explanation seems logical, since the calf fails to develop and grow normally on an all-liquid diet after a certain age *(1, 17)*, probably because of a shortage of volatile fatty acids and/or other necessary metabolites. Inasmuch as insulin fails to produce typical hypoglycemic convulsions and coma in the adult ruminant *(2)*, it is possible that the metabolic requirements, as well as the metabolic pathways in nervous tissue of the adult, may differ from those in the young calf. Further investigation of these relationships would seem desirable.

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AUREOMYCIN AS A PROTEIN-SPARING AGENT AND ITS INFLUENCE ON MINIMUM STARTER PROTEIN LEVEL SATISFACTORY FOR NORMAL GROWTH OF DAIRY CALVES

J. P. EVERETT, JR.,[1] L. D. BROWN,[1] C. A. LASSITER,[1]
D. R. JACOBSON, AND J. W. RUST
Dairy Section, University of Kentucky, Lexington

SUMMARY

Sixty two-day-old Holstein and Jersey calves were raised to 86 days of age on a limited milk-hay-starter system to determine *(a)* whether aureomycin spared dietary protein, and *(b)* the minimum starter protein level satisfactory for normal growth of aureomycin-fed calves reared on a limited milk-hay-starter system. Five starters ranging from 6.3 to 14.2% protein were fed with and without aureomycin. The antibiotic did not spare dietary protein in this experiment, but did give satisfactory gains on a starter containing at least 2% less protein than the starter required to give similar gains without aureomycin. Normal weight gains were made by calves on the 12.2 and 14.2% protein starters when aureomycin was fed. Significant increases in average daily gain, height at withers, heart girth, and starter consumption were obtained when aureomycin was fed. The antibiotic had no significant effect on apparent digestibility of any of the nutrients in the ration, nitrogen retention, blood levels of plasma protein, urea nitrogen or nonprotein nitrogen; however, aureomycin tended to lower slightly the blood levels of urea and nonprotein nitrogen. Aureomycin gave a slight but nonsignificant depression in rectal temperature. Calves on the 12.2 and 14.2% protein starters had significantly higher coefficients of apparent digestibility of crude protein, and the 14.2% protein groups had significantly greater nitrogen retention than did calves on the 6.3% protein starters.

Numerous studies have shown that aureomycin increases body weight gains of dairy calves reared under normal herd conditions *(9)*. The exact mode of action of this growth stimulant, however, remains an enigma. Several workers have suggested that antibiotics could possibly stimulate the growth of swine *(4, 8)* and poultry *(15)* by sparing crude protein in the diet.

Jensen *et al. (8)* proposed that the following criteria would have to be met to demonstrate a dietary sparing of protein by antibiotics: *(a)* the animals must show a greater growth response to antibiotics at low levels than at high protein levels, *(b)* there should be a significant protein × antibiotic interaction, and *(c)* antibiotics should improve nitrogen retention at any protein level, especially low-protein levels.

The primary objective of this study was to determine whether feeding aureomycin spared crude protein in the diet of young calves. In addition, the experiment was designed to supplement previous studies *(2)* at this station, by determining the minimum starter protein level satisfactory for normal weight gains of dairy calves reared on a limited milk-hay-starter system with aureomycin. This system, outlined subsequently, is an economical method of rearing herd replacements.

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[1] Present address: Dairy Dept., Michigan State University, East Lansing.

EXPERIMENTAL PROCEDURE

Sixty Holstein and Jersey calves were placed on a 5 × 2 factorial experiment utilizing five calf starters of varying protein content with and without aureomycin. Three calves of each breed were assigned to each of the ten resulting calf starters, the ingredients and chemical composition of which are listed (Table 1). Starters designated "A" contained 15 mg. of aureomycin per pound

TABLE 1
Composition of calf starters

Ingredients	Hay	Starter 1 and 1A	Starter 2 and 2A	Starter 3 and 3A	Starter 4 and 4A	Starter 5 and 5A
		(lb.)				
Ground yellow corn		350	505	410	514	379
Wheat bran		25	50	100	100	155
Ground oats		30	50	200	225	250
Linseed oil meal		10	10	15	20	35
Soybean oil meal		10	15	15	20	35
Corn distillers solubles		20	25	35	50	75
Molasses		50	50	50	50	50
Iodized salt		10	10	10	10	10
Steamed bone meal		10	10	10	10	10
Vitamins A and D supplements[a]		1	1	1	1	1
Corn starch		484	274	154	....	....
		1,000	1,000	1,000	1,000	1,000
		Chemical composition (%)				
Dry matter	88.4	89.8	88.8	88.9	87.5	88.8
Crude protein	13.1	6.3	8.6	10.1	12.2	14.2
Ether extract	2.3	1.8	2.4	3.3	4.1	4.1
Crude fiber	32.0	1.8	2.5	3.9	4.9	5.2
Ash	7.4	2.9	3.5	4.0	4.6	5.1
Nitrogen-free extract	33.6	77.0	72.0	67.6	61.7	60.2

[a] Containing 10,000 I.U. Vitamin A and 500 I.U. Vitamin D per g.

of feed. All starters were pelleted into $\frac{3}{16}$-in. pellets in an attempt to minimize variation in consumption due to differences in the physical texture of the starters. Since calves consume limited amounts of starter during the early weeks of age, animals on the aureomycin-containing starters ("A" starters) were fed 50 mg. of aureomycin daily in the milk to 49 days of age.

Calves were removed from their dams at two days of age, placed into individual stalls, and fed herd Holstein milk from nipple pails at the rate reported by Brown *et al. (3)* to 49 days of age. Calves were continued on experiment until 86 days of age. When placed on experiment, the animals were offered starter, medium-quality alfalfa-brome-grass hay, and water ad libitum with the exception of a daily limitation on starter consumption of 4 lb. for Jersey, and 5 lb. for Holstein, calves.

Each calf was weighed when placed on the experiment; subsequent body weights were taken weekly and at 43 and 86 days of age. Measurements of height at withers and heart girth were made at two, 43, and 86 days of age. Feed consumption and incidence of scours were recorded daily.

One Holstein and one Jersey calf from each of the ten groups were used in digestion and nitrogen-balance studies at 5, 8, and 11 wk. of age (designated Periods 1, 2, and 3, respectively). Total fecal and urinary collections were made for five days following a two-day preliminary period. Holstein calves were fed 1.6, 3.0, and 4.0 lb. starter and 0.4, 1.0, and 2.0 lb. hay daily during digestion Periods 1 through 3, respectively. Jersey calves were fed 1.2, 2.4, and 3.0 lb. starter and 0.3, 0.8, and 1.5 lb. hay during similar periods. Milk was fed only during the first digestion period. Chemical analyses of feed, and of fecal and urine samples, were made by A.O.A.C. methods *(1)*.

Jugular blood samples were obtained from two Jersey and two Holstein calves in each group at the beginning of the experiment and at 2-wk. intervals subsequently. Plasma protein nitrogen, plasma nonprotein nitrogen, and blood urea nitrogen determinations were made on the day of sampling according to standard methods *(6)*. Rectal temperatures of one calf from each breed on each of the ten experimental starters were taken at the time the animals were placed on trial and subsequently at 4 P.M. on Mondays, Wednesdays, and Fridays of each week throughout the trial.

In the statistical treatment of experimental data, analysis of variance was used and, if significant differences were indicated, this was followed by Hartley's multiple range test, as outlined by Snedecor *(13)*, to determine among which protein levels and starter groups these differences existed.

RESULTS

Growth. Aureomycin significantly increased daily gains and average increases in height at withers and heart girth (Table 2). With the exception of the weight gains of Group 1A, aureomycin at each protein level gave average daily gains and average increases in height at withers greater than the next higher protein level without the antibiotic. When aureomycin was fed, calves on the 12.2 and 14.2% protein starters made gains equal to those calculated from Morrison's 1956 standards *(11)* for animals of this age. The failure of aureomycin to stimulate growth of Group 3A apparently was due to the poor growth of one Jersey calf, which made an average daily gain of 0.54 lb.

When these data were considered by protein levels, an increase in average daily gains and height at withers was noted for each increase in protein content of the starter. Increase in heart girth showed generally the same trend with the exception of the 12.2% protein groups. Average values obtained by protein level are not due to protein per se, since aureomycin was fed to one-half of the calves on each protein level.

The percentage of protein in the starter had no significant effect on average daily gains of the calves to 6 wk. of age. This would be expected, since the calves received milk during this period. Aureomycin, however, significantly ($P<0.01$) increased average daily gains during this period. Holsteins gained significantly ($P<0.01$) faster than Jerseys during both the 6- and 12-week periods.

TABLE 2

Effect of protein level and aureomycin on growth of dairy calves from two to 86 days of age

Starters	Av. daily gain		Av. increase in Height at withers		Av. increase in Heart girth	
	(lb.)		*(in.)*		*(in.)*	
1	0.65		3.50		5.92	
1A	0.77		4.29		6.71	
Av. for 6.3% C.P.		0.71		3.90		6.31
2	0.79		3.96		7.46	
2A	1.04		4.92		8.50	
Av. for 8.6% C.P.		0.92		4.44		7.98
3	1.01		4.62		8.75	
3A	1.02		4.58		8.21	
Av. for 10.1% C.P.		1.02		4.60		8.48
4	0.95		4.38		7.38	
4A	1.25		5.29		9.29	
Av. for 12.2% C.P.		1.10		4.83		8.33
5	1.12		5.21		9.92	
5A	1.32		5.83		10.54	
Av. for 14.2% C.P.		1.22		5.52		10.23
No aureomycin (30 calves)	0.91[a]		4.33[a]		7.88[b]	
Aureomycin (30 calves)	1.08		4.98		8.65	

[a] $P<0.01$, [b] $P<0.05$.

Significant differences in average daily gain:
Protein levels: $P<0.01$—14, 12, 10%>6%; 14%>8%: $P<0.05$—8%>6%; 14%>10%.
Starters: $P<0.01$—5, 5A, 4A>1; 5A, 4A>1A, 2: $P<0.05$—4, 3, 3A, 2A>1; 5>1A, 2; 5A>4.

Significant differences in increase in height at withers:
Protein levels: $P<0.01$—14%>10, 8, 6%: $P<0.05$—14%>12%; 12%>6%.
Starters: $P<0.01$—5, 5A, 4A>1; 5A>2: $P<0.05$—2A>1; 4A>2; 5A>1A.

Significant differences in increase in heart girth:
Protein levels: $P<0.01$—14, 12, 10, 8%>6%; 14%>12, 10, 8%.
Starters: $P<0.01$—4A, 5, 5A>1; 5, 5A>1A; 5A>4, 2: $P<0.05$—2A, 3>1; 4A>1; 5>4,2.

Feed consumption and efficiency. Starter consumption increased generally (Table 3) with increasing protein levels, although calves in the 10.1% protein groups consumed less than calves in the 8.6% protein groups. Only the 14.2% protein groups ate significantly ($P<0.01$) more starter than the 6.3% groups. When considered by starter groups, aureomycin-stimulated starter consumption on every protein level with the exception of Group 3A. Group 5A consumed significantly ($P<0.01$) larger quantities of starter than did calves in Group 1; differences in starter consumption among other groups were not significant. Holstein calves consumed significantly ($P<0.01$) more starter than Jerseys.

Differences in the consumption of milk and hay were not statistically significant. The low hay consumption of Group 2 was due to two calves which consumed an average of 9.3 lb. per calf during the 84-day trial.

Increasing the protein level of the starter significantly improved the efficiency of feed utilization (Table 3). Aureomycin appeared to improve feed efficiency on each protein level, but this effect was not significant statistically.

Digestion coefficients and nitrogen balance. In the three digestion trials, the apparent digestibility of only the crude protein fraction was significantly influenced by starter protein level. Since aureomycin had no significant effect on

TABLE 3

Effect of protein level and aureomycin on feed consumption and utilization of the experimental calves

Starter	Average total consumption per calf						Feed/gain
	Milk		Starter		Hay		
	(lb.)						*(lb/lb)*
1	225.2		122.2		40.2		3.52
1A	217.3		145.3		47.9		3.48
Av. for 6.3% C.P.		221.3		133.7		44.0	3.50
2	214.0		147.2		28.8		3.13
2A	226.3		167.4		63.4		2.95
Av. for 8.6% C.P.		220.1		157.3		46.1	3.04
3	220.2		158.0		65.9		3.00
3A	218.3		153.8		53.6		2.74
Av. for 10.1% C.P.		219.3		155.9		59.7	2.87
4	205.3		155.7		45.2		2.96
4A	225.6		179.4		51.0		2.46
Av. for 12.2% C.P.		215.5		167.6		48.1	2.71
5	206.6		169.8		55.6		2.69
5A	217.0		198.4		49.5		2.50
Av. for 14.2% C.P.		211.8		184.1		52.6	2.60
No aureomycin (30 calves)	214.3		150.6[a]		47.1		3.06
Aureomycin (30 calves)	220.9		168.9		53.0		2.82

Significant differences in starter consumption:
Aureomycin: [a] $P<0.05$.
Protein levels: 14%>6% ($P<0.01$).
Starters: 5A>1 ($P<0.01$).
Significant differences in feed efficiency:
Protein levels: 14, 12, 10%>6% ($P<0.01$); 8%>6% ($P<0.05$).
Starters: 4A, 5A>1, 1A ($P<0.05$).

the digestibility of any of the nutrient fractions, only the apparent digestibility of crude protein is presented by periods (Table 4). Coefficients for other nutrient fractions are listed as averages for the three digestibility periods (Table 5). Complete digestibility data can be found in the thesis *(5)* from which this paper was taken.

The apparent digestibility of crude protein by calves on the 6.3% protein starters was significantly ($P<0.05$) lower than for calves on the 12.2 and 14.2% protein starters. However, crude protein digestibility was similar for all groups in the first digestion period when the calves were fed milk. The apparent digestibility coefficients of all nutrient fractions with the exception of crude fiber were significantly higher during Period 1 than in Periods 2 and 3.

Average daily nitrogen retention by calves on the ten experimental starters is presented as an average of the three nitrogen balance periods (Table 5) and by separate periods (Table 6). Aureomycin had no significant effect on nitrogen retention. However, calves in the 14.2% protein groups retained significantly ($P<0.01$) more nitrogen than calves in the 6.3% protein groups. The amounts of nitrogen retained by all calves were significantly ($P<0.01$) greater during Period 3 than in previous periods, and significantly greater ($P<0.05$) during Period 2 than in Period 1.

Blood urea nitrogen, plasma protein, and nonprotein nitrogen. Aureomycin depressed blood levels of urea nitrogen and nonprotein nitrogen, and increased blood plasma protein levels slightly, but none of these effects was statistically

TABLE 4

Average coefficients of apparent digestibility of crude protein of calves at 5, 8, and 11 wk. of age

Group	Period					
	1		2		3	
	(%)					
1	74.8		56.7		42.3	
1A	74.6		59.8		67.2	
Av. for 6.3% C.P.		74.7		58.2		54.8
2	80.9		64.1		67.0	
2A	76.4		62.8		68.4	
Av. for 8.6% C.P.		78.7		63.4		67.7
3	72.3		61.5		65.9	
3A	77.6		68.8		67.6	
Av. for 10.1% C.P.		75.0		65.2		66.8
4	79.7		70.8		67.1	
4A	78.4		69.2		71.4	
Av. for 12.2% C.P.		79.1		70.0		69.3
5	77.0		71.2		72.0	
5A	78.6		70.4		72.3	
Av. for 14.2% C.P.		77.8		70.8		72.2
No aureomycin (ten calves)	76.9		64.9		62.9	
Aureomycin (ten calves)	77.2		66.2		69.4	

Significant differences:
Periods: 1>2, 3 ($P<0.01$).
Protein levels: 14, 12%>6% ($P<0.05$).
Starters: 5, 5A, 4, 4A>1 ($P<0.05$).

significant. Protein level of the starter had no significant effect on plasma protein levels, in spite of the low-protein starters fed to some of the animals. However, calves in the 12.2% protein groups, but not the 14.2% groups, were found to have significantly ($P<0.05$) higher average blood urea nitrogen levels than calves in the 6.3% protein groups. There is no apparent explanation for this anomalous response of the 14.2% groups. Blood urea nitrogen, plasma protein,

TABLE 5

Apparent digestibility of all nutrient fractions and nitrogen retention averaged for all periods

Starter group	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Nitrogen retention
	(%)					(g/day)
1	75.8	57.9	72.6	33.8	86.0	3.5
1A	73.5	67.2	80.2	40.9	78.2	11.0
Av. for 6.3%	74.6	62.6	76.4	37.3	82.1	7.2
2	80.2	70.7	82.2	49.8	86.5	10.0
2A	79.0	69.2	84.1	47.0	85.6	10.9
Av. for 8.6%	79.6	69.9	83.1	48.4	86.0	10.4
3	76.2	66.6	84.9	42.6	83.8	13.3
3A	77.3	71.3	86.6	47.6	83.3	7.7
Av. for 10.1%	77.0	69.0	85.8	45.1	83.6	10.5
4	77.9	72.6	89.4	52.9	83.7	9.7
4A	77.9	73.0	89.8	47.8	84.2	11.0
Av. for 12.2%	77.9	72.8	89.6	50.0	83.9	10.4
5	76.2	73.4	86.3	40.6	84.3	16.2
5A	75.5	73.8	87.3	42.4	82.2	14.6
Av. for 14.2%	75.8	73.6	86.8	41.4	83.2	15.4
No aureomycin	77.2	68.2	83.1	43.9	84.8	10.5
Aureomycin	76.7	70.9	85.6	45.0	82.7	11.0

TABLE 6

Average daily nitrogen intake and retention of calves during the three nitrogen balance periods

Group	Period 1			Period 2			Period 3		
	N intake	N Balance		N intake	N Balance		N intake	N Balance	
	(g.)	*(g.)*	*(% of dig. N)*	*(g.)*	*(g.)*	*(% of dig. N)*	*(g.)*	*(g.)*	*(% of dig. N)*
1	10.8	0.2	3.7	17.4	6.8	68.6	19.5	3.6	14.0
1A	16.2	9.4	78.3	19.0	7.2	62.2	32.6	16.5	75.9
2	12.7	3.1	23.6	21.8	10.0	71.6	33.9	16.9	74.8
2A	19.1	9.7	68.8	23.0	8.2	58.2	30.1	14.8	71.8
3	18.1	8.8	55.2	27.7	11.9	70.6	38.6	19.3	75.5
3A	12.9	3.0	10.4	24.3	11.9	71.0	26.6	8.1	45.8
4	17.9	9.3	65.3	25.4	12.0	67.0	26.2	7.9	42.7
4A	18.4	7.0	34.4	26.6	13.1	73.4	39.3	13.0	43.0
5	24.1	12.1	65.8	36.6	16.5	63.8	46.9	20.0	59.2
5A	22.5	8.4	46.6	36.6	14.6	55.0	52.9	20.8	50.6
No aureomycin	16.7	6.7	42.7	25.8	11.4	68.0	33.0	13.5	53.2
Aureomycin	17.8	7.5	47.7	25.9	11.0	63.9	36.4	14.6	57.4
6.3% C. P.	13.5	4.8	41.0	18.2	7.0	65.4	26.0	10.0	45.0
8.6% C. P.	15.9	6.4	46.2	22.4	9.1	64.9	32.3	15.8	73.3
10.1% C. P.	15.5	5.9	32.8	26.0	11.9	70.8	32.6	13.7	60.6
12.2% C. P.	18.2	8.2	49.8	26.0	12.6	70.2	32.8	10.4	42.8
14.2% C. P.	23.3	10.2	56.2	36.6	15.6	59.4	49.9	20.4	54.9

Significant differences in nitrogen balance (g.):
Protein level: 14%>6% ($P<0.01$).
Starters: 5>1 ($P<0.01$); 5A, 3>1 ($P<0.05$).
Periods: 3>2, 1 ($P<0.01$); 2>1 ($P<0.05$).

and nonprotein nitrogen values decreased with increasing age of the calves during the first 8 wk. of the trial. This change possibly could be influenced either by age or by dietary change or by both.

Rectal temperatures and scours. It has been suggested *(10)* that the growth-promoting effect of aureomycin resulted from suppression of subclinical infections. An attempt was made in this study to detect subclinical infections by rectal temperatures. Such infections, if present, were not detected by this technique.

The average 12-wk. rectal temperature was 102.6° F. for calves receiving aureomycin and 102.8° F. for the controls. This slight depression of rectal temperature was not significant, and these observations fail to support, with dairy calves, the results of a recent trial with sheep *(14)*, in which aureomycin caused a significant depression of rectal temperature.

The incidence of scours was extremely low. Aureomycin-fed calves averaged 0.87 scouring day per calf and the controls, 0.80 day per calf during the 84-day experimental period.

DISCUSSION

Using the criteria listed previously for the primary objective, aureomycin did not spare dietary protein in this study. Aureomycin failed *(a)* to give a greater growth response at the lower protein levels than at higher protein levels, *(b)* to give a significant protein × antibiotic interaction, and *(c)* to significantly improve nitrogen retention.

Aureomycin increased body weight gains of calves 18.5% on the low-protein starter and 17.8% on the high-protein starter. The possibility that the 14.2% protein level used in this study was not high enough to demonstrate a protein-sparing effect has been considered. However, in view of the report *(12)* that aureomycin increased by 20% the growth of Jersey and Holstein calves fed a 19.4% digestible protein starter, it is unlikely that this antibiotic would have given a reduced stimulation had higher protein levels been used in the present study.

Nitrogen retention for aureomycin-fed animals was 11.0 g. per day, while that of the controls was 10.5 g. per day. This slight difference was not statistically significant and agrees with the results reported by Lassiter *et al. (10)* but not with those of Hogue *et al. (7)*.

Although the effect of aureomycin on nitrogen retention was not statistically significant, the low-protein group receiving the antibiotic exhibited an improved utilization of absorbed nitrogen in Periods 1 and 3. However, this apparent difference is probably influenced to a greater extent by the poor appetite of the low-protein group controls while in the digestion crates.

To determine the starter protein level satisfactory for normal growth of aureomycin-fed calves reared under the conditions of this experiment, the secondary objective of this study, average daily gain, was considered the most sensitive response criterion.

Even though aureomycin did not spare dietary protein, the antibiotic-fed calves made satisfactory gains to 86 day of age on a starter containing at least

2% less crude protein than the starter required to produce similar gains when aureomycin was omitted from the ration. Both the 12.2 and the 14.2% protein starters gave normal growth when aureomycin was fed. These gains compare favorably with those of previous studies at this station, in which Holstein and Jersey calves made average daily gains of 1.00 and 1.30 lb. *(2)* on starters containing approximately 16.0% crude protein. The greater body weight gains of the antibiotic groups were probably, in part, the result of increased dry matter consumption, but the cause of this stimulation was not apparent in this study.

Nitrogen balance was considered to be less sensitive than average daily gain in evaluating the protein level satisfactory for calves fed aureomycin, since a smaller number of calves was used and feed acceptance was variable when the calves were confined in digestion crates. The data, however, indicate that at least the 14.2% protein level was satisfactory for calves reared under the system used in this study. The observation that nitrogen retention increases with age agrees with other reports *(2, 3, 7)*. In the present study, this increase could have been influenced also by dietary change.

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INFLUENCE OF ANTIBIOTICS ON THE GROWTH AND PROTEIN METABOLISM OF YOUNG DAIRY CALVES

C. A. LASSITER, R. M. GRIMES, AND C. W. DUNCAN
Departments of Dairy and Agricultural Chemistry,
Michigan State University, East Lansing

SUMMARY

Twenty-one two-day-old calves were used to evaluate the effect of aureomycin and bacitracin on growth and protein metabolism. Calves fed rations containing either antibiotic tended to gain at a faster rate, consumed more calf starter and total feed, and made better utilization of their feed (feed per pound of gain) than did the control calves. None of these differences was statistically significant. Neither antibiotic appeared to reduce the incidence of scours. Both antibiotics significantly lowered the coefficients of dry matter and crude fiber digestibility, but neither antibiotic appeared to have any great effect on the digestibility of other nutrients or on nitrogen retention. Aureomycin significantly increased blood serum levels of total protein, while bacitracin significantly decreased them; but neither had an appreciable effect on blood levels of urea or nonprotein nitrogen. Both antibiotics significantly increased the relative serum concentration of albumin and lowered the concentration of alpha-globulin, but had little effect on beta-globulin concentration. Calves fed both antibiotics tended to have lower levels of serum gamma-globulin than did the control calves.

The results indicate that these antibiotics had little effect on over-all digestion and protein metabolism of the calf, suggesting that the mode of action in stimulating the growth of calves does not appear to be through an effect on protein metabolism or on digestibility.

It has been well established that certain antibiotics improve the growth rate, feed utilization, and general health of young dairy calves, but the mode of action of antibiotics has not been established. Many of these studies have been reviewed by Knodt *(6)*, Lassiter *(7)*, and Reid *(15)*.

In an early report, Murley *et al. (13)* observed that when calves were fed a restricted diet aureomycin produced a slight but not a significant improvement in nitrogen utilization. Lassiter *et al. (10)* failed to find that aureomycin had any significant effect on the digestion of calves. In this study, aureomycin had no effect on the blood level of urea nitrogen but did cause a significant depression in the level of blood nonprotein nitrogen during the first 7 wk. of the calf's life. In a later study *(9)*, these workers found that neither aureomycin nor terramycin, either in the crystalline or supplement form, had any significant effect on the digestion of feed nutrients or the retention of nitrogen. Blood studies indicated that neither antibiotic had any effect on the blood levels of urea nitrogen or total nonprotein nitrogen. Logan *et al. (11)* reported that feeding aureomycin did not affect the digestion of young calves, and that a positive growth response was not obtained. Pritchard *et al. (12)* observed that aureomycin feeding had practically no effect on the digestibility of dry matter, protein, crude fiber, fat, ash, or nitrogen-free extract of rations fed to 8-wk.-old

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calves. The work of Bartley *et al. (1)* confirmed the earlier reports, that the beneficial effect of aureomycin on the growth of calves was not due to an improvement in digestibility of the rations. Recent work by Everett *et al. (4)* showed that aureomycin had no significant effect on the apparent digestibility of any nutrient, nitrogen retention, the level of plasma protein, urea or nonprotein nitrogen, although there was a tendency toward slightly lower levels of urea and nonprotein nitrogen.

Contrary to these negative reports, Hogue *et al. (5)* observed from digestion trials with calves conducted at 7 and 16 wk. of age that aureomycin significantly increased the apparent digestibility of crude protein and nitrogen retention. The average protein digestibility, as well as nitrogen retention, was higher for bacitracin-fed calves than the control calves, but these differences were not statistically significant. Neither antibiotic had a significant effect on the digestibility of other nutrients.

Dhopeshwarkar *et al. (3)* observed that the administration of antibiotics to human beings significantly lowered the blood level of gamma-globulin.

The present study was initiated to study further the influence of antibiotics on the growth rate, protein metabolism, and certain nitrogenous blood constituents of the young dairy calf.

EXPERIMENTAL PROCEDURE

Twenty-one two-day-old male calves were divided into three comparable groups and raised to 86 days of age on a milk replacer-starter-hay system. Five large breed and two small breed calves composed each group. All calves were fed a basal milk replacer composed of the following poundages: dried skimmilk—52.4, cerelose—30.0, corn distillers dried solubles—10.0, dried whey—5.0, dicalcium phosphate—2.0, salt containing cobalt and iodine—0.5, and a vitamin and mineral mix [2]—0.1., according to the feeding schedule shown in Table 1. Group 1

TABLE 1

Daily milk replacer feeding schedule

Age	Small breeds		Large breeds	
	Milk	Replacer	Milk	Replacer
(days)	*(lb.)*			
0– 2	Dam		Dam	
3–10	4.0	0.4	4.0	0.6
11–21	....	1.0	....	1.4
22–28	....	0.8	....	1.0
29–42	....	0.6	....	0.8
43–49	....	0.4	....	0.6
50–over	Off replacer			

calves were used as the controls; Groups 2 and 3 received the basal milk replacer supplemented with either crystalline aureomycin or bacitracin at the rate of 50 mg. of antibiotic per pound of milk replacer. All calves were fed a basal calf

[2] Noller *et al. (14)*.

starter composed of these poundages: ground yellow corn—732.5, crimped oats—627, linseed oil meal—133, soybean oil meal—133, corn distillers dried solubles—200, cane molasses—133, dicalcium phosphate—20, trace mineral salt—20, and a vitamin concentrate [3]—1.5. Group 1 calves were fed the basal starter, and Groups 2 and 3 were fed the basal starter supplemented with 15 mg. per pound of either crystalline aureomycin or bacitracin. Aureomycin and bacitracin were the antibiotics selected for use in this study, since most work *(7)* indicated that aureomycin produces a maximum growth response in calves; whereas, bacitracin usually produces a minimum response. Calf starter and alfalfa hay feeding were begun at 1 wk. of age. Hay was fed free choice, but large-breed calves were limited to 5 lb. of calf starter daily and small-breed calves to 4 lb.

Digestion and nitrogen balance trials were conducted with each calf at approximately 5, 8, and 11 wk. of age. The amount of feed fed during each of the digestion trials is shown (Table 2). The total amount of feed fed during

TABLE 2

Feed consumed during digestion trials [a]

Trial No.	Small breeds			Large breeds		
	Replacer	Starter	Hay	Replacer	Starter	Hay
1	0.6	1.0	0.3	0.8	1.5	0.5
2	....	1.8	0.6	....	2.5	0.8
3	....	3.0	1.0		3.9	1.3

[a] (lb/day/calf).

the trials increased with age, but the ratio of starter to hay was held constant. The feed intake of the calves was standardized for three days prior to a five-day collection of feces and urine. Venous blood samples were taken from each calf at 2, 4, 6, 8, 10, and 12 wk. of age. The samples were analyzed for blood urea nitrogen by the urease method, serum nonprotein nitrogen by digestion of a protein-free filtrate followed by steam distillation and nesslerization, total serum protein by the Kjeldahl method, and the relative percentage of serum albumin, alpha-, beta-, and gamma-globulin by paper electrophoresis. The apparatus used was a Spinco Model R and the amount of serum used was 10 μl. A barbital buffer at pH 9.0 and ionic strength 0.1 was employed. The duration of the electrophoresis was 16 hr. at 10 Ma. at room temperature. Staining was done by the method of Block *et al. (2)* and quantitatively evaluated by an automatic recording and integrating densitometer.

RESULTS AND DISCUSSION

The influence of the two antibiotics on the growth rate of the calves is shown in Table 3. Both antibiotics improved the rate of gain and skeletal growth of the calves. These differences approached statistical significance at both 7 and 12 wk. of age. In this study, the growth stimulation from aureomycin and bacitracin was similar. The rate of gain for all calves was slightly subnormal, probably due to the involvement of the calves in the metabolism trials.

[3] 10,000 I.U. of vitamin A and 1,000 I.U. of vitamin D per gram.

TABLE 3
Effect of crystalline aureomycin and bacitracin on the growth of young dairy calves

Group	Average [a] starting weight	Average daily gain [a]		Average increase [a]	
		0–7 wk.	0–12 wk.	Ht. at withers	Chest circumference
(No.)	*(lb.)*			*(in.)*	
1—Control	82.7	0.50	0.81	3.29	5.54
2—Aureomycin	89.6	0.67	0.98	4.29	7.68
3—Bacitracin	89.7	0.71	0.97	4.11	6.79

[a] No significant differences among groups.

As shown in Table 4, both antibiotics increased the consumption of starter and total feed and improved the efficiency of feed conversion. None of these differences among groups was significant. Neither antibiotic appeared to have any pronounced influence on the incidence of scours. This is difficult to explain,

TABLE 4
Influence of aureomycin and bacitracin on feed consumption, feed efficiency, and incidence of scours of young dairy calves

Group	Average total consumption [a]				Feed/lb gain	Incidence of scours [a]
	Milk	Replacer	Starter	Hay		
(No.)	*(lb.)*					*(days/calf)*
1—Control	32.0	39.2	150.5	35.2	3.37	5.4
2—Aureomycin	31.7	39.2	166.5	34.5	2.96	6.1
3—Bacitracin	32.0	39.3	162.5	45.9	3.09	4.4

[a] No significant differences among groups.

in view of numerous reports *(7)* indicating a reduction of calf scours by antibiotic feeding. Lassiter *et al. (9)* observed that crystalline aureomycin had a less beneficial effect on both the rate of gain and incidence of scours in calves than when the aureomycin supplement, Aurofac, was fed. Other trials *(8)* with antibiotics, in which an aureomycin supplement was included, were in progress at the same time as this study which showed that aureomycin significantly increased the growth rate of calves and drastically reduced the incidence of calf scours. Based on these observations, it would appear that additional evidence has been obtained to indicate that aureomycin supplements may contain factors other than aureomycin which promote growth and have a beneficial effect on the incidence of calf scours.

Digestion and nitrogen balance data (Table 5) indicate that both antibiotics significantly decreased the digestibility of dry matter and crude fiber, but had little effect on the digestibility of ether extract or nitrogen-free extract. Bacitracin tended to reduce the digestibility of crude protein more than did aureomycin.

Disregarding rations, the digestibility of all nutrients, with the exception of crude fiber, tended to decrease with age of the calves. Values for dry matter digestibility were 81.8, 78.2, and 76.0% for Trials 1, 2, and 3, respectively. Values

TABLE 5

Effect of aureomycin and bacitracin on the digestion and metabolism of young dairy calves

	Group			L.S.D.	
	Control	Aureomycin	Bacitracin	5%	1%
	Coefficients of apparent digestibility				
Dry matter	80.2	78.1	76.7	1.6	....
Crude protein	75.3	75.0	72.1	n.s.	....
Ether extract	80.9	77.4	78.3	....	....
Crude fiber	61.7	49.2	47.8	5.0	6.6
Nitrogen-free extract	86.6	84.8	84.4	....	....
	Nitrogen balance *(g/day)*				
Dry matter intake	1,174.2	1,253.2	1,271.0	n.s.	....
Nitrogen intake	35.5	37.8	38.3	n.s.	....
Fecal excretion	8.8	9.6	10.5	0.8	....
Urinary excretion	14.9	14.1	14.8	n.s.	....
Total excretion	23.7	23.7	25.3	n.s.	....
Nitrogen retained	11.8	14.1	13.0	n.s.	....
Per cent retained	33.2	37.3	33.9	n.s.	....

for crude protein were 76.2, 72.7, and 73.5%, for crude fiber, 52.5, 55.7, and 50.5% for Trials 1, 2, and 3, respectively.

Both antibiotics, but particularly bacitracin, significantly increased the excretion of nitrogen in the feces although they had little effect on excretion

TABLE 6

Influence of aureomycin and bacitracin on certain blood constituents

	Group		
Age	1	2	3
(wk.)	Blood urea nitrogen *(mg. %)*		
2	14.4	9.8	11.8
4	13.1	10.5	12.2
6	9.6	11.0	8.6
8	15.0	12.8	14.0
10	10.5	9.4	11.5
12	9.2	12.1	11.2
Average [a]	12.0	10.9	11.6
	Nonprotein nitrogen *(mg. %)*		
2	27.0	29.7	26.4
4	25.1	23.0	23.4
6	21.1	23.2	21.4
8	28.7	27.6	31.2
10	23.0	24.7	24.1
12	23.9	23.8	24.4
Average [a]	24.8	25.3	25.2
	Total serum protein *(g/100 ml.)*		
2	6.09	6.45	5.68
4	5.85	6.13	5.46
6	5.75	6.24	5.44
8	6.10	6.82	5.70
10	6.39	6.40	6.08
12	6.75	6.43	6.79
Average [b]	6.16	6.41	5.86

[a] No significant differences among groups.
[b] 1% L.S.D.—0.16.

of nitrogen in the urine or on total nitrogen excretion. Aureomycin and bacitracin tended to increase total nitrogen retention, but only aureomycin improved the per cent nitrogen retained of the total intake. None of these differences was statistically significant. These data confirm other reports which show that antibiotics have little effect on protein digestibility and nitrogen retention *(1, 4, 9, 10–13)*, but they are not in agreement with the report by Hogue *et al. (5)*. Nitrogen retention increased with age of the calves, averaging 8.9, 10.0, and 20.0/g per day for Trials 1, 2, and 3, respectively, when the data are combined for all rations.

Neither antibiotic appeared to have any marked effect on the level of blood urea nitrogen or nonprotein nitrogen (Table 6). However, aureomycin significantly increased the level of total serum protein, whereas bacitracin had a similarly depressing effect. An explanation of this phenomenon is not evident.

TABLE 7

Influence of aureomycin and bacitracin on the relative concentration of various blood proteins

Age	Group 1	2	3
(wk.)		Albumin *(%)*	
2	34.4	35.6	37.9
4	36.7	38.1	40.7
6	35.8	38.9	39.4
8	39.2	40.3	41.7
10	32.9	39.9	40.0
12	33.9	40.1	39.3
Average [a]	35.5	38.8	39.8
		Alpha-globulin *(%)*	
2	22.3	21.5	22.6
4	23.2	20.5	21.4
6	22.0	19.0	21.2
8	20.7	18.2	20.5
10	22.1	19.3	21.2
12	22.1	19.1	20.9
Average [b]	22.1	19.6	21.3
		Beta-globulin *(%)*	
2	22.1	20.4	21.5
4	19.9	19.9	20.4
6	20.3	20.9	19.1
8	18.9	20.6	18.1
10	20.4	19.3	18.2
12	20.2	19.6	19.6
Average [c]	20.3	20.1	19.5
		Gamma-globulin *(%)*	
2	21.2	22.6	17.9
4	20.1	21.5	17.6
6	22.4	20.4	20.2
8	21.1	20.9	19.6
10	24.7	21.4	20.8
12	23.8	20.3	20.2
Average [c]	22.2	21.2	19.4

[a] 1% L.S.D.—0.54.
[b] 1% L.S.D.—1.12.
[c] No significant differences among groups.

Blood samples were taken before the calves were placed on experiment, but the values were so erratic that they were not presented.

As shown in Table 7, both antibiotics significantly increased the relative percentage of serum albumin, tended to decrease the alpha-globulin, but had little effect on the beta-globulin. The antibiotics tended to decrease the relative concentration of gamma-globulin, a decrease which approached significance at the 5% level of probability. This study was initiated primarily to study the effect of antibiotics on the relative concentration of serum gamma-globulin, in view of the work of Dhopeshwarkar *et al. (3)*. These data possibly indicate that the mode of action of antibiotics in calves is through a reduction in disease level, thus causing a reduction in the serum gamma-globulin fraction. Although the data are not presented, the calves of the smaller breeds tend to have a lower relative concentration of serum albumin and alpha-globulin, and a higher level of serum beta- and gamma-globulins, than calves of the larger breeds.

The results of this study show that aureomycin and bacitracin have little effect on the protein metabolism of young dairy calves. The possibility exists that the techniques and criteria used were not exacting enough to detect any influence the antibiotics may have on protein metabolism. With the possible exception of the tendency for the antibiotics to reduce the concentration of serum gamma-globulin, which is probably the result of the lower disease level, there was no evidence obtained which showed that the mode of action of antibiotics in calves is through an effect on over-all digestion or protein metabolism.

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EFFECT OF PROTEIN LEVEL IN CALF STARTERS ON THE GROWTH RATE AND METABOLISM OF YOUNG CALVES

L. D. BROWN,[1] C. A. LASSITER,[1] J. P. EVERETT, JR.,[1] D. M. SEATH, AND J. W. RUST
Dairy Section, University of Kentucky, Lexington

SUMMARY

Eighty two-day-old male and female calves representing the Holstein and Jersey breeds were used in two separate experiments, to determine the effect of protein level of calf starters on growth rate and metabolism of young dairy calves.

In the first experiment, no significant differences were observed in growth rates of calves receiving starters containing either 12.2, 16.6, 20.2, or 24.3% crude protein. The most efficient gains made were on the 12.2 and 16.6% protein levels. In the second experiment, no significant differences were found among groups receiving either a 13.0, 20.0, or 23.7% crude protein starter. A starter containing only 8.5% crude protein supported growth at a significantly lower rate than any of the other starters. A 16.6% crude protein starter gave growth superior to any of the other groups, which may be explained in part by increased feed consumption. The most efficient gains as measured in terms of pounds of feed required per pound of gain were made by this group. The digestibility, nitrogen balance, and blood studies tended to confirm results obtained from growth studies.

Based on these studies, it appears that dairy calves two to 84 days of age do not require starters containing more than 12.0 to 16.0% crude protein, to maintain normal growth when a limited amount of whole milk and a medium to good quality alfalfa hay are fed. It was also shown that starters containing only 8.5% crude protein would not support normal growth, thereby indicating that the minimum protein level of calf starters is in excess of 8.5% crude protein.

During the past several years, considerable research has been conducted *(2, 3, 4, 6, 11, 12)* to determine the optimum protein level of rations for swine and poultry. Results of studies in both fields have led to recommendations of lower protein rations. Some limited work *(8, 9, 10)* of this nature has been conducted using 3- to 4-mo.-old calves in which similar growth was obtained from feeding both high- and low-protein rations.

In general, high-protein feeds are more expensive and, therefore, it is economically desirable to feed only the amount of protein required to maintain normal performance of the animals in question.

The purpose of this study was to determine the minimum protein level of calf starters satisfactory for normal growth of calves raised on a limited milk-hay-starter system.

The results reported cover two separate experiments, and will be designated as Experiments I and II.

EXPERIMENT I

Procedure. Forty two-day-old male and female calves were selected at random and divided into four similar groups based on breed and body weight. Each group, composed of five Jersey and five Holstein calves, was raised to 86 days

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[1] Present address: Dairy Department, Michigan State University, East Lansing.

of age on a limited milk-hay-starter system. The milk-feeding schedule was the same as outlined in a previous report from this station *(1)*. The experimental starters (Table 1) were fed in the meal form with a maximum daily limit of 4 lb. for Jerseys and 5 lb. for Holsteins. A medium quality alfalfa-brome-grass hay was fed ad libitum.

TABLE 1

Composition of calf starters (Experiment 1)

Ingredients	Starter			
	1	2	3	4
	(lb.)			
Ground yellow corn	78	178	279	423
Crimped oats	112	264	418	640
Wheat bran	46	111	175	77
Linseed oil meal	335	230	125	35
Corn distillers solubles	670	460	250	70
Iodized salt	15	15	15	15
Bone meal	15	15	15	15
Vitamins A and D supplement *(g.)*[a]	600	600	600	600
Molasses	65	65	65	65
	1,337	1,339	1,343	1,341

	Hay	Chemical composition			
		(%)			
Dry matter	92.0	91.3	91.9	89.8	90.7
Crude protein	13.4	24.3	20.2	16.6	12.2
Ether extract	2.4	5.5	5.3	4.7	4.1
Crude fiber	34.2	5.9	6.1	5.6	6.3
N.F.E.	35.3	46.6	52.2	56.6	63.3
Ash	6.7	9.0	8.1	6.3	4.8

[a] 10,000 I.U. vitamin A and 500 I.U. vitamin D per gram.

Each calf was weighed at two days of age and at weekly intervals throughout the 12-wk. experimental period. Measurements of chest circumference and height at withers were taken at two and at 86 days of age. Records of feed consumption, incidence of scours, and general condition of the calves were made daily.

Digestion and nitrogen balance studies were conducted using five male calves (three Holsteins and two Jerseys) from each of the four groups at the ages of 5, 8, and 11 wk. Feces and urine were quantitatively collected for five consecutive days. The feeding regime for calves in the digestion crates was altered to correspond with the ratios of grain-to-hay of 4 : 1, 3 : 1, and 2 : 1 at 5, 8, and 11 wk. of age, respectively. Blood samples were collected from the same calves at 1, 3, 5, 7, 9, and 11 wk. of age. Blood serum protein determinations were made immediately after collection according to standard methods *(5, 7)*.

The four experimental starters used in this study contained identical ingredients, varying only in the amounts of each ingredient so as to obtain the desired protein level. As shown in Table 1, Starters 1, 2, 3, and 4 contained 24.3, 20.2, 16.6, and 12.2% crude protein, respectively.

Results. The effect of protein level in the starter on average daily gains and on increases in height at withers and heart girth is presented (Table 2). The

TABLE 2

Effect of level of protein on average growth, feed consumption, and blood levels of serum protein of young dairy calves to 84 days of age

	Starter group			
	1	2	3	4
Growth[a]				
Initial weight *(lb.)*	72.5	73.8	73.2	73.9
Daily gain *(lb.)*	0.84	0.92	1.00	0.98
Increase in height at withers *(in.)*	3.42	3.95	4.18	4.45
Increase in heart girth *(in.)*	6.15	6.85	7.30	7.75
Feed consumption[a]				
Milk *(lb.)*	196.7	203.5	200.6	206.2
Starter *(lb.)*	138.1	159.3	159.2	159.6
Hay *(lb.)*	56.9	62.4	59.2	52.5
Feed efficiency *(lb. feed/lb gain)*[b]	3.12	3.21	2.91	2.89
Blood serum protein *(g/100 ml.)*[a]				
Age *(wk.)*				
1	5.67	5.14	4.98	6.05
3	5.57	5.74	5.46	5.65
5	5.70	5.36	5.85	6.05
7	5.92	6.04	6.10	6.26
9	6.68	6.38	6.94	6.40
11	6.30	6.71	6.70	7.01
Average	5.97	5.90	6.00	6.24

[a] No significant differences among groups.
[b] L.S.D. ($P < 0.05$) = 0.13; ($P < 0.01$) = 0.18.

average daily gains were not significantly different as determined by analysis of covariance *(16)*. Since these gains were somewhat below the average for similarly aged calves at this station, it should be pointed out that five calves from each group were used in the metabolism studies for a period of 3 wk. during the 12-wk. experimental period. It was observed that even though feed consumption was not interrupted seriously, the calves gained somewhat less rapidly during the periods spent in the metabolism units. The gain of calves in Starter Groups 2, 3, and 4 was equal to Ragsdale's Growth Standards *(15)*. The differences among groups in average increase in heart girth and height at withers were not statistically significant.

The average consumption of milk, starter, and hay, and the amount of total feed required per pound of gain for each of the four groups, are presented (Table 2). The differences in average consumption of starter were not statistically significant, even though the starter consumption of the calves on Starter 1 was approximately 21 lb. less than for the other three groups. This can be explained in part by the fact that two Jerseys and one Holstein calf on this starter consumed only 81.7, 97.8, and 125.0 lb., respectively. The physical texture of this starter could have been a contributing factor to its decreased consumption, since it contained large amounts of linseed oil meal and distillers solubles, which resulted in a fine-textured feed.

A highly significant difference ($P < 0.01$) was found among starter groups as to the efficiency of feed utilization. Starter Groups 3 and 4 required significantly less feed per pound of gain than Starter Groups 1 and 2. There was no significant difference between Starter Groups 1 and 2 or 3 and 4.

The average coefficients of apparent digestibility and daily nitrogen retention values are presented (Table 3). These values are representative of the diet as a whole rather than the individual starters per se. The digestibility of protein was significantly lower ($P < 0.01$) for Starter Group 4 than for the other experimental groups. The small differences in dry matter and crude fiber digestibility among groups were not significant. Dry matter and crude protein coefficients of digestibility decreased with age of the calf, regardless of the ration. Crude fiber coefficients of digestibility remained about the same, even though the ratio of hay to grain increased with the age of the calf.

TABLE 3

Average coefficients of apparent digestibility and daily nitrogen retention

	Starter group			
	1	2	3	4
Nitrogen balance *(g.)*				
Dry matter intake *(average for all periods)*	1,204	1,403	1,473	1,603
Nitrogen intake *(average for all periods)*	44.8	45.2	42.0	36.4
Nitrogen in feces *(average for all periods)*	11.2	11.3	11.0	11.7
Nitrogen in urine *(average for all periods)*	18.2	18.3	14.6	10.9
Total nitrogen excreted	29.4	29.6	25.6	22.6
Nitrogen retained	15.4	15.6	16.4	13.8
Period 1	8.5	11.1	10.4	7.8
Period 2	16.9	15.4	19.0	15.0
Period 3	20.9	20.5	19.6	18.7
Per cent nitrogen retained of intake	34.4	34.5	39.0	37.9
Apparent digestibility *(%)*				
Dry matter *(average for all periods)*	75.8	77.1	78.1	75.4
Period 1	80.6	80.8	83.4	79.9
Period 2	74.7	76.0	76.6	74.1
Period 3	72.3	74.5	74.3	72.3
Crude protein *(average for all periods)*[a]	75.7	75.7	75.0	68.7
Period 1	80.4	79.4	80.8	74.7
Period 2	74.6	74.7	73.1	65.9
Period 3	72.2	73.0	71.0	65.5
Crude fiber *(average for all periods)*	49.9	56.3	56.2	53.9
Period 1	50.0	55.6	56.0	54.4
Period 2	48.2	55.9	55.9	53.6
Period 3	51.6	57.5	56.8	53.8

[a] L.S.D. ($P < 0.05$) = 2.86; ($P < 0.01$) = 3.82.

Average daily nitrogen retention values of 15.4, 15.6, 16.4, and 13.8 g. for Starter Groups 1, 2, 3, and 4, respectively, were not significantly different. When based on per cent of nitrogen intake, the trend was for slightly higher nitrogen utilization by calves on Starters 3 and 4 than for those on Starters 1 and 2.

The five calves from each of the four experimental groups used in the metabolism studies were also used to determine the effect of protein level in the ration on the blood level of serum protein. The small differences observed (Table 2) between the weekly averages of the calves on each protein level were not significant. The calves on the lowest protein starter (Group 4) had significantly lower coefficients of apparent digestibility of crude protein, but consistently had the highest blood serum protein levels. Indication was found of a relationship between the serum protein level and the age of the calf, with the trend being for

higher levels as the calves increased in age. Based on these data, there was no correlation between the level of protein in the starter and the serum protein level of the blood.

EXPERIMENT II

Procedure. Forty two-day-old male and female calves representing the Holstein and Jersey breeds were divided into five similar groups based on breed and body weight. Each group, composed of four Jersey and four Holstein calves, was raised to 86 days of age on a limited milk-hay-starter system. The feeding and management of the calves were essentially the same as outlined in Experiment I. The protein levels of the starters in this experiment ranged from 8.5 to 23.7% crude protein (Table 4). Since a difference in starter consumption had been noted in the first experiment, probably due to physical texture, all of the starters in this experiment were made into 3/16-in. pellets. Hay from the same lot was fed during both experiments.

TABLE 4
Composition of calf starters (Experiment II)

Ingredients	Starter				
	1	2	3	4	5
	(lb.)				
Ground yellow corn	297	382	466	843	585
Crimped oats	278	360	440	360	216
Wheat bran	187	240	292	200	24
Linseed oil meal	270	160	52	24	24
Soybean oil meal	294	184	76	19	19
Corn distillers solubles	160	160	160	40	40
Iodized salt	16	16	16	16	16
Bone meal	16	16	16	16	16
Vitamins A and D supplement (g.)[a]	726	726	726	726	726
Molasses	80	80	80	80	80
Corn starch	.....	.....	.....	.....	578
Total	1,600	1,600	1,600	1,600	1,600

	Hay	Chemical composition				
		(%)				
Dry matter	88.4	88.6	89.8	89.0	89.2	88.9
Crude protein	13.1	23.7	20.0	16.2	13.0	8.5
Ether extract	2.3	3.7	3.8	4.2	3.8	2.5
Crude fiber	32.0	6.4	6.0	5.5	5.1	2.8
N.F.E.	33.6	48.4	53.8	57.4	62.6	71.5
Ash	7.4	6.4	6.2	5.7	4.7	3.6

[a] 10,000 I.U. vitamin A and 500 I.U. vitamin D per gram.

Results. The average daily gains and increases in height at withers and heart girth are given (Table 5). When statistically treated by the analysis of variance *(16)*, it was found that Starter Group 3 had a significantly higher ($P < 0.05$) average daily gain than any of the other four groups. This can be explained, in part, on the basis of increased feed consumption by this group. Another possible explanation is that of an optimum protein-energy ratio existing in Starter 3. No significant differences were found among Starter Groups 1, 2, and 4; however,

all groups gained in body weight significantly faster ($P < 0.01$) and increased significantly more in heart girth than Starter Group 5. The small differences among groups in increase in height at withers were not significant.

The feed consumption and feed efficiency data are presented (Table 5). Groups 1 and 3 consumed significantly more ($P < 0.01$) starter than Group 5. It also was found that Group 3 consumed significantly more starter ($P < 0.05$) than Groups 2 and 4. No other significant differences were found among groups in starter consumption. The small differences in hay consumption among groups were not significant.

TABLE 5

Effect of level of protein on average growth, feed consumption, and blood plasma protein levels of young dairy calves to 84 days of age

	Starter group					L.S.D.	
	1	2	3	4	5	(P<0.05)	(P<0.01)
Growth							
Initial weight *(lb.)*	73.8	74.5	73.5	72.8	75.0		
Daily gain *(lb.)*	1.12	1.12	1.30	1.02	0.79	0.17	0.23
Increase in height at withers *(in.)*	5.28	4.84	4.97	4.81	4.19		
Increase in heart girth *(in.)*	8.47	8.97	9.53	8.25	6.62	1.42	1.90
Feed consumption							
Milk *(lb.)*	216.9	208.8	221.9	199.8	209.7		
Starter *(lb.)*	172.6	151.9	186.6	154.5	132.2	27.5	36.9
Hay *(lb.)*	40.7	42.7	43.0	45.0	31.6		
Feed efficiency *(lb. feed/lb gain)*	2.59	2.40	2.37	2.63	2.87	0.22	0.30
Blood plasma protein *(g/100 ml.)*[a]							
Age *(wk.)*							
0	7.22	5.95	6.40	6.24	5.80		
2	6.35	5.60	6.09	5.92	5.14		
4	5.79	5.30	5.76	5.42	4.76		
6	5.69	5.28	5.39	5.85	5.49		
8	5.63	5.07	5.53	5.88	5.29		
10	5.74	5.51	5.33	5.39	5.65		
12	5.45	6.02	5.88	5.59	5.29		
Average	5.98	5.53	5.77	5.76	5.34		

[a] No significant differences among groups.

Wide variations in feed efficiency existed among the five experimental groups. Starter Groups 1 and 4 required significantly more feed per pound of gain than Group 3 ($P < 0.05$) and Group 4 required significantly more than Group 2 ($P < 0.05$). However, all groups exhibited significantly higher feed efficiency values than did Starter Group 5.

The average coefficients of apparent digestibility and daily nitrogen retention values are presented (Table 6). In agreement with results of Experiment I, the differences in nitrogen retention among Starter Groups 1, 2, 3, and 4 were not significant; however, Groups 2, 3, and 4 retained significantly more nitrogen ($P < 0.05$) than did Starter Group 5. There was also a significant period difference, with the calves retaining significantly more nitrogen ($P < 0.01$) during Period 3 (11 wk.) than in Periods 1 and 2 (5 and 8 wk. of age, respectively).

TABLE 6

Average coefficients of apparent digestibility and daily nitrogen retention

	Starter group					L.S.D.	
	1	2	3	4	5	(P<0.01)	(P<0.05)
Nitrogen balance *(g.)*							
Dry matter intake *(average for all periods)*	1,373	1,228	1,349	1,402	1,190		
Nitrogen intake *(average for all periods)*	53.5	41.4	38.2	33.9	22.4		
Nitrogen in feces *(average for all periods)*	11.5	9.0	9.3	9.0	7.7		
Nitrogen in urine *(average for all periods)*	28.6	15.0	13.6	9.5	4.7		
Total nitrogen excreted	40.1	24.0	22.9	18.5	12.4		
Nitrogen retained	13.4	17.4	15.3	15.4	10.0	.....	4.31
Period 1	10.6	13.4	11.0	7.2	6.3		
Period 2	11.5	14.8	10.7	16.1	10.4		
Period 3	18.1	24.1	24.1	23.0	13.2		
Per cent nitrogen retained of intake	25.0	42.0	40.0	45.4	44.6		
Apparent digestibility *(%)*							
Dry matter *(average for all periods*	74.6	77.4	77.2	79.0	80.3	3.56	2.67
Period 1	76.9	81.5	81.4	81.2	82.0		
Period 2	72.6	75.2	74.7	76.9	79.4		
Period 3	74.4	75.6	75.7	79.0	79.6		
Crude protein *(average for all periods)*	78.6	78.9	76.0	74.2	66.8	4.28	3.21
Period 1	79.6	83.5	80.4	77.5	74.8		
Period 2	77.8	75.9	72.6	70.2	63.5		
Period 3	78.2	77.2	75.0	75.0	62.2		
Crude fiber *(average for all periods)*	47.8	48.9	51.2	55.3	50.8		
Period 1	43.0	42.4	49.6	52.7	40.9		
Period 2	48.3	49.3	49.6	53.1	50.5		
Period 3	52.0	55.1	54.6	60.1	61.2		

The average fecal nitrogen excretions were similar among the five experimental groups. However, considerable variation was noted among groups as to the amount of nitrogen excreted in the urine. The average urinary nitrogen was highest for Starter Group 1, followed in descending order by Starter Groups 2, 3, 4, and 5. This apparent trend suggests an excess of protein in the high-protein starters. Since the TDN values were relatively close for the five experimental starters, protein contributed a somewhat greater portion of the total energy in the higher protein starters. In agreement with observations made in the previous study, Starter Group 4 had a significantly lower apparent digestibility of crude protein ($P < 0.01$) than did Starter Groups 1 and 2. However, all groups had significantly higher coefficients of crude protein digestibility than Starter Group 5 ($P < 0.01$). No appreciable differences were found among groups in crude fiber digestibility.

The effect of protein level in the starter on the blood plasma protein level was found to be not significant among starter groups. This is in agreement with observations made in the previous experiment.

DISCUSSION

In both experiments, it was observed that calves receiving starters containing approximately 16% crude protein gained slightly more than calves receiving either higher or lower protein starters. This difference can not be explained solely on the basis of increased feed consumption and may be indicative of a particular protein-energy ratio being desirable in calf starters. Since all of the starters contained approximately equal amounts of TDN, the protein-energy ratios varied accordingly with the protein level of the starter.

In Experiment II, the relative growth rates of the five experimental groups for the first 6 wk. of life were very similar. From 6 wk. of age to 12 wk. of age, calves in Group 5 increased approximately 42 lb. as compared with increases averaging 65 lb. for Starter Groups 1, 2, and 4, with Starter Group 3 increasing approximately 72 lb. It appears that the limited amount of whole milk fed for the first 6 wk. was sufficient to maintain normal growth, irrespective of starter fed. However, when the milk feeding rate was lowered to 2% of body weight (6 wk. of age) it was necessary for calves to obtain required protein in sufficient amounts from other sources. In this case, all animals were able to maintain their growth rate with the exception of calves fed the low-protein starter (8.5% crude protein). The general growth trends obtained for the first 6 wk. vs. growth for the last 6 wk. for calves on normal and deficient rations is in agreement with a previous report from this station *(1)*.

The average daily dry matter intake of calves used in the nitrogen balance studies (Table 6) on the five experimental starters was quite similar. Since Starter 4 was the lowest protein starter (13.0% C.P.) to maintain normal growth, this group was used to compare protein intakes with that of accepted standards. At 8 wk. of age (average body weight, 112.8 lb.) the calves receiving Starter 4 had a daily intake of 0.27 lb. of digestible protein per 100 lb. of body weight as compared with Morrison *(13)* and N.R.C. *(14)* standards of 0.30 and 0.40 lb., respectively. At 12 wk. of age (average weight, 155.1 lb.), however, the daily intake had increased to 0.31 lb. of digestible protein per 100 lb. of body weight, which meets the minimum standards as outlined by Morrison *(13)*, but remains slightly below N.R.C. standards *(14)*.

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EFFECT OF A MIXTURE OF HIGH-SOLIDS REMADE SKIMMILK AND COLOSTRUM ON YOUNG CALVES [1]

J. M. WING
Department of Dairy Science,
Florida Agricultural Experiment Station, Gainesville

SUMMARY

Six pairs of newborn calves were used in a 60-day trial to study the effects of a mixture of colostrum and skimmilk containing 20% solids. The high-solids group gained an average of 70 lb. as compared to 54 lb. for controls which received skimmilk containing 13% solids. Calves in the high-solids group consumed a total of 171 lb. of concentrate feed as compared to 353 lb. eaten by the controls. These differences are significant at $P = 0.01$.

The high-solids calves gained an average of 4.2 in. in height at withers and controls gained an average of 3.8 in. The high-solids calves consumed a total of 94.4 lb. hay and the controls consumed 63.0 lb. No scouring was observed in controls, and two calf-days of abnormal feces were observed in the high-solids group.

Considerable effort has been devoted to the development of systems for raising calves using a minimum of whole milk. Various workers have shown that colostrum could replace a considerable part of the marketable milk usually fed to calves *(1–5)*. In herds where all calves are raised and all colostrum is used, enough pure colostrum is available to feed the calves for less than 2 wk.—a period which is too short to replace all the whole milk. Gaunya *et al. (2)* found a mixture of colostrum and water to compare favorably with whole milk for calves to 35 days of age, though colostrum alone appeared to be diarrheic. Keyes *et al. (4)* reported satisfactory results, however, when colostrum was fed intermittently with whole milk, and Parrish and coworkers *(6)* found the constituents of colostrum to be highly digestible. In a 60-day trial, Jacobson *et al. (3)* found that colostrum compared favorably with whole milk, but that it did not prevent scours. Wise and La Master *(14)* found that a mixture of reconstituted skimmilk and colostrum was satisfactory for calf feeds. They suggested that the mixture for older calves should be not over 50% colostrum.

Use of high-solids skimmilk is an inexpensive way to insure a high dry matter intake in young calves, but milks containing more than 13% solids-not-fat are likely to be diarrheic *(13)*. This objection, though not eliminated, was partially corrected by use of supplementary chlortetracycline *(6)*.

The present experiment was undertaken to observe the effects of a mixture of colostrum and high-solids skimmilk in the diet of young calves. The criteria employed included body weights, heights at withers, efficiency of feed utilization, incidence of scours, and general appearance.

EXPERIMENTAL PROCEDURE

Twelve calves of Holstein, Jersey, and Guernsey breeds born during the spring of 1958 served as experimental subjects. All calves were removed from

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their dams at birth and confined to individual pens inside a closed barn. Pangola-grass hay and dry mixed concentrates [2] were supplied free choice. During the first four days all calves were fed fresh colostrum from nipple pails.

On the afternoon of the fourth day postpartum, all calves were weighed and measured to determine heights at withers. The calves were paired at this time on the basis of body weight, height at withers, breed, sex, and general appearance.

Colostrum used was a composite of the first four days' milk from Jersey, Guernsey, and Holstein cows. When a surplus accumulated, it was frozen and the amount required for immediate use was thawed prior to each feeding. Milk was fed to all subjects according to the following schedule: one through four days, pure colostrum; five through 21 days, one-half colostrum and one-half skimmilk; 22 through 60 days, skimmilk. The skimmilk for one member of each pair (control) contained 13% by weight of air-dry solids. Pair-mates received skimmilk reconstituted at the rate of 20% air-dry solids. The daily milk allowance of 9% of body weight was computed at weekly intervals and fed in two equal portions daily. Each calf received 15,000 I.U. vitamin A [3] daily to eliminate the possible deficiency of this vitamin in the controls.

Solid feed remaining in the boxes was removed once daily and replaced with a fresh offering. A record of the amount consumed was made at this time. All calves were examined thrice daily for abnormalities, particularly diarrhea.

Weekly records were maintained on body weights and heights at withers. At the end of 60 days all records were terminated and data were analyzed statistically, using the method described by Snedecor *(9)* for paired data.

RESULTS AND DISCUSSION

General environmental conditions for this experiment were especially favorable for general health and growth. All calves were thrifty, as shown by smooth coats, clear eyes, alertness, and good appetites. Calves in the high-solids group were larger, but there were no other indications that they were healthier than the controls.

Two calves in the high-solids group scoured for one day each. These digestive disturbances were mild and no treatment or change in the feed was necessary.

Average growth in body weight usually expected of normal animals similar to the calves used in this work is 47 lb., as computed by use of the Ragsdale standard *(8)*. This compares to a group mean gain of 54 lb. by the control calves. The group average gain in weight for calves which received high-solids milk was 70 lb. (Table 1). This trend was consistent with all pairs and, statistically, the difference is significant at $P = 0.01$.

Increases in height at withers also were more pronounced in the treated group, which gained an average of 4.2 in. compared to a mean gain of 3.8 in. by the controls. This difference was not statistically significant.

[2] By weight in parts: ground shelled corn, 300; ground oats, 250; wheat bran, 100; cottonseed meal (41%), 130; nonfat dry milk solids, 100; common salt, 8.5, and steamed bone meal, 17.

[3] Nopcay 250 supplied by NOPCO Chemical Company, Harrison, New Jersey.

TABLE 1

Physical response of calves to colostrum and high-solids skimmilk

	Control							High solids						
	Calf No.							Calf No.						
	1	3	5	7	9	11	Average	2	4	6	8	10	12	Average
Initial body weight *(lb.)*	85	64	63	53	54	72	65	82	64	60	53	53	74	64
Gain in body weight *(lb.)*	75	60	49	49	47	46	54	106	71	65	57	60	59	70
Initial height *(in.)*	29.1	28.0	27.2	25.2	26.0	29.0	27.4	30.0	26.8	26.0	25.6	26.6	29.1	27.2
Gain in height *(in.)*	3.9	3.9	3.1	3.9	4.3	3.5	3.8	4.7	4.3	3.5	3.9	3.9	4.7	4.2
Days abnormal feces observed	0	0	0	0	0	0	0	1	0	1	0	0	0	

High-solids milk appeared to discourage consumption of concentrate feeds. During the course of the experiment, the control calves consumed 353 lb. of dry mixed concentrates, while the high-solids calves ate only 171.9 lb. This difference is statistically significant at the 1% level. The opposite trend was observed with respect to tendency to consume hay. The control group ate 63.0 lb., and the high-solids group consumed 94.4 lb. (Table 2). This difference, however, was not statistically significant. Appetite appeared to be affected largely with respect to type of feed and rate of growth.

All changes in type of milk fed were abrupt and no treatment was used in

TABLE 2

Total consumption of dry feeds weekly by groups of calves

Group	Type of dry feed	Weeks on experiment								
		1	2	3	4	5	6	7	8	Total
		(lb.)								
Control (6 calves)	Hay	1.2	1.9	3.0	10.3	8.7	10.2	12.7	15.0	63.0
	Concentrate	11.6	25.3	38.4	43.2	53.2	62.0	67.0	52.3	353.0
High solids (6 calves)	Hay	2.1	3.2	3.9	9.1	12.3	16.3	23.9	23.6	94.4
	Concentrates	6.8	11.9	13.0	21.3	29.3	26.6	32.3	30.7	171.9

event of scours. It seems remarkable that digestive disturbances were almost entirely absent in calves fed high-solids milk.

It also seems remarkable that a group of newborn calves with a mean weight of 64 lb. gained an average of 70 lb. each in 56 days. This gain is almost 30% more than that made by comparable controls. This consistently faster growth in the experimental group, as well as habits of solid-feed consumption, was similar to the response of young calves to dietary orotic acid and methionine *(12)*. Since dry milk solids contain approximately 0.6 mg. orotic acid per gram *(11)*, this may have been a factor. It is possible, however, that the difference was due entirely to a higher TDN intake by the high-solids calves.

While the results presented herein are not fully explained, they do emphasize the practicality of an extended colostral period, with or without high-solids milk, as an extremely simple means of eliminating all marketable milk from calf rations.

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USE OF HIGH-NITROGEN FEEDSTUFF MADE FROM WHEY IN THE RATIONS OF DAIRY ANIMALS [1,2]

D. G. HAZZARD,[3] E. M. KESLER, D. R. ARNOTT,[4] AND STUART PATTON

Department of Dairy Science, The Pennsylvania State University, University Park

SUMMARY

Feeding trials have been conducted to evaluate a high-nitrogen whey derivative as a feedstuff for dairy cattle. Preliminary trials indicated that the product was not toxic and appeared to support growth of 6-mo.-old calves. In one trial, two groups of eight lactating Holstein cows were used in a single-reversal experiment consisting of two 8-wk. periods. All cows were fed grass silage, timothy hay, and a concentrate mixture according to body weight and milk production. The experimental variable consisted of the presence or absence of the whey product in the concentrate mixture at the 10% level. Milk and butterfat production by cows fed the whey product was lower than that of controls, especially during the first period of the experiment. While receiving the whey product, the cows lost significantly more body weight than while on the control ration. Feed refusals by cows fed the whey product were significantly greater than those of control cows, and nutrient intakes of cows fed whey products were below requirements, due to the unpalatable nature of the product. Variations between individuals were noted, with regard to acceptance. It is believed that the portion of the feedstuff actually consumed was well-utilized. No significant differences were noted in blood glucose or blood lactic acid concentrations. In a second trial, 15 male Holstein calves were fed from six days to 20 wk. of age on calf starters which contained 0, 5, or 8% of the whey product. Growth by the three groups of calves was similar, those receiving the 5% level weighing slightly more at 20 wk. than did the other two groups. Consumption of the 5% starter was good, but the 8% mixture apparently lacked in palatability.

A process has been developed for the manufacture of a high-nitrogen, low-lactose material from whey *(1)*. Due to the nature of the reactions involved, it is believed that a major constituent of the product is ammonium lactate. It is commonly known that ruminants can utilize nonprotein nitrogen (NPN) as a part of the ration. While most research has been conducted with urea as the source of NPN *(9)*, other materials such as dicyandiamide and various ammoniated products have proven satisfactory *(4, 6, 7)*. In 1951, Seekles *(10)* reported that ammonium lactate was beneficial in the treatment of bovine ketosis. In later work, Shaw *et al. (11)* found that other lactates also were beneficial for treating ketosis. Hueter *et al. (5)* noted that marked increases in blood levels of lactic acid and glucose occurred as a result of direct absorption of lactic acid from the rumen.

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[2] Taken from a thesis submitted by the senior author in partial fulfillment of requirements for the M.S. degree.

[3] Present address: Department of Animal Industries, University of Connecticut, Storrs.

[4] Present address: Ontario (Canada) Agricultural College, Guelph.

The present study was initiated to determine whether the whey product *(1)* has value as a constituent of the ration of dairy cows and calves.

EXPERIMENTAL PROCEDURE

Preliminary investigation had indicated that lactating cows could be fed concentrates which contained low levels of the whey product with no apparent deleterious effects. In further preliminary work, three 6-mo.-old male Holstein calves were used to test the product for toxicity. One calf served as a control while the other two were fed concentrates which contained the whey product at an initial level of 10% on a weight basis. After one week at this level, the percentage was increased to 15, 20, 30, and 40% during successive weeks. The whey product had a total solids level of 72.7%. Mechanical problems in mixing and storage arose due to high moisture content, when levels of incorporation exceeded 15%. All calves received 6 lb. of concentrate and 5–8 lb. of hay per head daily. It was estimated that when on the higher level the animals were receiving over 50% of their daily protein requirements from the whey product. The calves grew normally and gained in excess of 2 lb. body weight daily during the 5-wk. period. No toxicity symptoms were noted, although one calf did exhibit diuresis at the higher levels of whey product intake. An initial palatability problem was encountered with one calf; however, after being forced by hunger to consume the ration, the individual thereafter ate it without objection. Additional preliminary observations were made in attempts to overcome the problem of poor palatability of the whey product. It was found that when the concentrate portion of the ration contained levels of the whey product not in excess of 10% it could be fed successfully if incorporated into grass silage and the two fed together. This procedure was adhered to in the subsequent feeding trial with cows.

TRIAL I. WHEY PRODUCT TO MILKING COWS

Animals and feeding. Sixteen lactating Holstein cows were divided into two similar groups on the basis of milk production, body weight, age, and stage of lactation. They were utilized in a single-reversal experiment to test the value of the whey product as an ingredient of the concentrate mixture fed for milk production. The experiment consisted of two 56-day periods, each preceded by an adjustment period of 1 wk.

The groups were fed similarly except for inclusion of the whey product in the experimental concentrate mixture. The roughage consisted of late-cut timothy hay and grass-legume silage, fed at levels of approximately 0.75 lb. of hay and 4.0 lb. of silage per 100 lb. body weight. Both hay and silage were fed twice daily. Composition of the concentrate mixtures used is given (Table 1). The experimental mix contained 10% of whey product, substituted in place of molasses and soybean oil meal. This necessitated a higher level of ground corn to obtain equal calculated TDN levels in the two mixtures. For purposes of calculation, assumed values of 50.0% crude protein and 65.0% TND were used for the whey product. The average composition of the whey product as fed is given in Table 1 (footnote). As prepared, some batches contained total solids

TABLE 1

Concentrate mixtures containing whey product, fed to cows and calves

Ingredient	Trial I—Lactating cows		Trial II—Calves		
	Control	10% Whey product	I Control	II 5% Whey product	III 8% Whey product
Corn[a]	700	860	900	1100	1220
Ground oats	750	750	500	500	500
Soybean oil meal	310	50	500	300	180
Whey product[b]	.....	200	.....	125	200
Wheat bran	100	100	350	350	350
Cane molasses	100	.....	200	75	.....
Iodized salt	20	20	25	25	25
Steamed bone meal	20	20	.....	.....	.....
Dicalcium phosphate	.....	.....	25	25	25
Vitamin A and D supplement	.....	.....	5	5	5
Total	2000	2000	2505	2505	2505

[a] Corn was ground for cows, cracked for calves.

[b] Average composition of whey product solids, in percentages: total nitrogen, 12.7; NPN, 10.8; ash, 7.6; lactose, 8.2; fat, 1.5; lactic acid, 48.6; other 10.6 *(1)*. Product was fed to cows at total solids level of 72.7; to calves, at 74.0.

above 72.7%, but were standardized to this solids content for use. The material was liquid and could be handled with molasses equipment for batch mixing. Concentrates were fed at rates calculated to supplement the roughages and meet the individual requirements for digestible protein and TDN according to the Morrison standard *(8)*. Adjustments in the amount of concentrates fed were made at biweekly intervals, using production of milk and butterfat for the preceding three days as the basis for calculation. Concentrates were fed with the silage to induce consumption. All feedstuffs offered were weighed and feed refusals weighed back.

All cows were housed and managed alike. The barn was a bank-type structure, artificially lighted and ventilated. The cows were individually stanchioned and wheat straw was used for bedding. Each animal had access to salt block and water bowl. The herd was turned outside for exercise daily.

Observations. Records were made of feed intake, milk and fat production, and body weight changes. Milk weights were recorded at each milking. Butterfat tests were made on A.M.–P.M. composite samples from each cow at biweekly intervals, using the Babcock test. All cows were weighed, on two successive days, at the beginning of the first and the end of the 5th and 8th wk. of each experimental period.

Blood samples were obtained from the jugular vein during the 2nd, 4th, and 8th wk. of each period. Potassium oxalate was used as anticoagulant. Samples were obtained approximately 4.5 hr. after the morning feeding. Protein-free filtrates of the blood samples were prepared, using barium hydroxide and zinc sulfate. Blood glucose and blood lactic acid were determined on the filtrates, using the Somogyi *(12)* and the Barker and Summerson *(2)* methods, respectively. It was necessary to freeze part of the filtrates until time became available to perform the analyses.

Organoleptic analyses of the milk produced by cows fed the experimental and control rations were conducted on three separate occasions. Flavor criticisms were noted, but a numerical system of scoring was not used.

TRIAL II. WHEY PRODUCT TO YOUNG CALVES

Animals and feeding. Male Holstein calves were assigned at random to three groups of five each, as follows: Group I—control, Group II—fed calf starter containing 5% whey product, Group III—fed starter containing 8% whey product. All calves weighed between 85 and 115 lb. and were between one and five days of age when brought to the experimental barn. They were obtained from neighboring farms, state institutional herds, and from the University Research herd. They were placed on experiment when six days old and remained thereon until 20 wk. of age. They were kept in individual stalls equipped with water bowls and salt block, in a barn which was artificially heated and ventilated. Wood shavings were used as bedding.

The calves were fed according to the limited milk-milk replacement-starter-hay system. All received colostrum for at least one day and 8 lb. whole milk daily until six days of age. Beginning on the sixth day, a commercial milk replacement was substituted gradually so that after ten days of age no milk was was fed. A total of 39.4 lb. of dry replacement, in 356 lb. warm water, was fed during the first 42 days of life, after which the calf subsisted on starter and hay. Second-cutting alfalfa–light grass hay was fed in ad libitum quantities throughout the trial. Any uneaten hay was weighed back once weekly. The starter mixture fed each group (Table 1) was offered in ad libitum amounts from six days of age until the individual was consuming 5 lb. daily, and this amount was fed thereafter. Starter was weighed back weekly or whenever it became soiled.

Observations. Initial measurements for body weight, height at withers, and chest circumference were made when each calf was six days of age. These measurements were repeated for all calves once weekly, and when the individual was 140 and 141 days of age. The latter measurements were averaged and used as final, 20-wk. data. The amounts of all feeds offered and refused were recorded.

RESULTS AND DISCUSSION

Milk production. Although the cows had been grouped on the basis of milk production, those fed the whey product (Group A) began the first experimental period at a somewhat lower level of production, due to a decline during the seven days of adjustment period which preceded. The whey product ration was not palatable and the cows refused large quantities during the adjustment period. Production by Group A continued at a lower rate during Period I, averaging 33.5 lb. per cow daily as compared to 39.2 lb. by the controls (Table 2). The whey-fed group declines in production more than did the controls. Butterfat production by the controls also was considerably higher than that of Group A, a reflection of the amounts of milk produced and of a slightly higher fat test. During Period II, after the groups were reversed, there were only negligible differences in production of milk and butterfat between controls and experimental animals

TABLE 2
Effect of feeding whey product to lactating cows[a]

	Period I (56 days)		Period II (56 days)	
	Group A fed whey product	Group B control ration	Group A control	Group B whey product
Milk production *(lb/day)*	33.5	39.2	25.2	25.7
Decline during period	12.0	9.4	5.7	12.5
Milk fat production *(lb/day)*	1.14	1.43	0.86	0.87
Change in body wt. *(lb.)*	−43	−32	+17	−58
Concentrate *(lb/day)*				
Offered	14.0	16.5	6.0	5.2
Refused	1.2	0.1	0.1	1.1
Mix[b] refused	0.9	0.2	0.0	1.8
Blood lactic acid *(mg/100 ml.)*	12.1 ±15.5[c]	6.3 ± 6.3	11.3 ±11.6	4.8 ± 3.1
Blood glucose *(mg/100 ml.)*	45.7 ±11.1	39.9 ±10.4	33.3 ± 4.0	31.4 ± 5.1

[a] Data based on eight cows per group. Blood lactic acid and glucose values are averages of three samples during each period.
[b] Mixture of concentrate and silage in which individual components could not be separated.
[c] Standard deviation.

(Table 2); however, during this period the whey-fed cows (Group B) declined 12.5 lb. per cow daily in milk production, as compared to a decline of 5.7 lb. by Group A. It should be noted that the method employed (wherein concentrate was fed in accordance with production) may have tended to penalize the Group A cows in Period II. However, they did gain in body weight; therefore, it is doubtful that they were underfed. The system of feeding concentrates was not the limiting factor for Group A cows in Period I, since they consistently refused some of the concentrate.

Analysis of variance for a cross-over design *(3)* indicated a highly significant difference in milk fat production due to treatment ($F = 15.9$ with one and 30 degrees of freedom). This was not true of milk production, due most likely to the lack of difference between groups in Period II.

Body weight changes. Both groups of cows lost weight during Period I; however, Group A, fed the whey product, lost more weight than did the control animals (Table 2). After the reversal of treatments at the beginning of Period II, Group A animals gained, and weighed only 26 lb. less at the end of the trial than at the beginning. Group B animals, switched to the whey product ration at the beginning of Period II, continued to lose weight during the period and at an accelerated rate. They weighed 90 lb. less at the end of the trial than at the beginning. An analysis of variance of body weight changes indicated a highly significant difference between treatments, verifying the excessive weight losses by cows while on whey product ration.

Feed consumption. During the entire trial, the cows fed whey product received less feed than did the controls, due to the system of feeding according to body weight and production. Differences were not great, however, with respect to hay and silage. In spite of being fed approximately 15% less concentrate,

the cows fed whey product refused a great deal more of it and of the silage with which it had been mixed (Table 2). There were marked individual differences between cows in acceptance of the concentrate containing whey product; a few individuals consumed their allotment with relish, while others refused a part of their feed about two-thirds of the times it was offered. The three cows which did not refuse the whey product feed declined in milk production much less than did the remainder of the groups while receiving the feed. At the same time, they lost very little body weight. These are possible indications that the whey product feed was utilized satisfactorily if consumed.

An exact calculation of nutrient intake was not made, due to the difficulties encountered as a result of mixing concentrate with silage in order to induce consumption of the whey product. The resultant weighback was a mixture on which exact proportions of concentrate and silage could not be determined. In addition, since no digestibility figures are available for the whey product, an assumed TDN content would have to be used. It does appear, however, that feed intake was the limiting factor relative to performance by cows while on the whey product ration.

Blood and milk substances. Blood samples from all cows were analyzed for lactic acid and glucose in an effort to determine whether the feeding of the whey product had an effect on blood levels of these metabolites. Samples were obtained at 2, 4, and 8 wk. after the beginning of each experimental period. Condensed results are presented (Table 2). Individual variations were noted between animals, but no significant differences existed due to treatment, with respect either to blood lactic acid or to blood glucose concentrations. Animals of Group B consistently were lower in blood lactic acid than were those of Group A. During the first weeks of the trial, both whey product and control fed animals showed an unexplainable decline in blood glucose levels.

The organoleptic grading of the milks produced by cows fed the two rations did not indicate any flavor attributed to the whey product feed. The flavors observed covered a normal range for milk collected during winter feeding practices.

TRIAL II

Growth and feed consumption. Growth, as measured by gain in body weight, height at withers, and chest circumference was as rapid in those calves fed the whey product as in the controls (Table 3). At 20 wk. of age, Group II calves,

TABLE 3

Effect of feeding whey product to Holstein calves

	Group I (Control)			Group II (5% Whey product in starter)			Group III (8% Whey product in starter)		
	Initial	12 wk.	20 wk.	Initial	12 wk.	20 wk.	Initial	12 wk.	20 wk.
Body weight *(lb.)*	101	207	327	99	215	353	103	200	334
Height at withers *(cm.)*	75	87	97	75	85	97	75	84	94
Chest circumference *(in.)*	31	38	44	31	39	46	31	38	46
Starter consumption *(lb.)*		195	445		192	471		112	341
Hay consumption *(lb.)*		83	354		96	407		138	471

fed 5% whey product, were an average of 26 lb. heavier than were controls. This difference was not statistically significant, due to individual variations and having only five calves per group. None of the other criteria of growth differed greatly between groups, and none was significantly different. The 12-wk. group data indicate that the whey product probably was utilized by the calves prior to this age.

Starter and hay consumption differences approached significance at the 5% level. It is apparent that the level of 8% whey product lacked in palatability, since consumption was lower and since the calves ate more hay, apparently in preference to the starter. Group II calves, fed 5% whey product, consumed slightly more starter than did controls. The data suggest that at this level palatability was not reduced and that the nutrients contained therein were utilized by calves. When compared to the cows, these calves had a relatively large intake of the whey product, on a body weight basis. It is suggested that if the palatability problem can be overcome the product may have value as a feed ingredient.

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DISTILLERS GRAINS, BREWERS GRAINS, AND UREA AS PROTEIN SUPPLEMENTS FOR DAIRY RATIONS

J. K. LOOSLI AND R. G. WARNER
Department of Animal Husbandry and Nutrition, Cornell University, Ithaca, N. Y.

SUMMARY

Two lactation experiments were conducted with 20 Holstein cows each, fed concentrate mixtures containing corn distillers dried grains, brewers dried grains, and urea as crude protein (nitrogen) supplements to a low-protein concentrate mixture. The protein-deficient basal ration resulted in weight loss, lower milk yields, and lower fat percentages than the other mixtures, and these changes were observed as early as 2 wk. after the cows were changed to the low-protein feed. The feeds containing corn distillers dried grains and brewers dried grains were superior to the low-protein feed with respect to total milk as well as fat-corrected milk yields. Urea was useful as a nitrogen source, but as an average was not so effective as the two other feeds. Corn distillers dried grains appeared to be somewhat more valuable than brewers dried grains.

In a series of studies with lactating cows, Loosli and Warner *(7, 8)* and Warner *et al. (17)* demonstrated that Holstein cows produced somewhat more milk when corn distillers dried grains were used as the primary protein supplement than when certain other protein sources were employed. More than 60 yr. ago, Hills *(5)* reported that cows produced more milk on corn distillers dried grains than on brewers dried grains, and that brewers dried grains were approximately equal to a mixture of linseed oil meal and cottonseed meal. More recent studies with brewers grains apparently have not been conducted in this country.

Urea has been tested as a partial substitute for protein supplements for milking cows, and it is now used to some extent; however, some dairymen still question its value. Controlled experiments, as reviewed by Reid *(13)*, suggest that urea may be slightly less valuable for milk production than true proteins, but there is no general agreement as to the level of intake which will give best results. Ward *et al. (16)* reported that urea and soybean oil meal were of comparable value for milk production. Earlier studies *(1, 2, 4, 6, 11, 12, 14, 15)* showed that cows often produced slightly less milk on urea than when equivalent nitrogen was fed as protein-containing feeds, but any difference was less at lower milk yields. In European countries, especially, urea has generally given less favorable results with cows than in the United States.

Experiments were planned to compare corn distillers dried grains with brewers dried grains and urea, as crude protein supplements in practical rations for high-producing dairy cows.

EXPERIMENTAL PROCEDURE

Two experiments were carried out, the first during the winter of 1955–56 and the second during the winter of 1956–57. In each experiment, 20 purebred Holstein cows were grouped into replicates of four cows each and randomly assigned to the four dietary treatments. The order of subsequent treatments

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was randomly determined in replicates of 4 × 4 Latin squares. The cows were fed all of the hay and silage they would eat, and records were kept of feeds consumed. The level of concentrates fed was established according to milk production during the pre-experimental period, and the amount fed was reduced at a uniform rate for all cows during the experiment *(9)*. The ingredients used in the concentrate mixtures are shown (Table 1), and the chemical analyses of the feeds are summarized (Table 2).

Individual records were kept of the milk produced daily. Fat percentage of the milk from each cow was determined each week on composite samples consist-

TABLE 1

Ingredients used in the concentrate mixtures

Ingredient	Pounds per 1,000 lb.[a]			
Yellow corn, ground	210	210	580	610
Oats, ground	200	200	200	200
Wheat bran	100	100	100	100
Corn distillers dried grains	400	.....	.....	.....
Brewers dried grains	.....	400	.....	.....
Urea	.....	.....	30	.....
Cane molasses	70	70	70	70
Dicalcium phosphate	10	10	10	10
Salt	10	10	10	10

[a] One gram of cobalt sulfate was added to each 1,000 lb.

TABLE 2

Chemical composition of the feeds used

Feed	Percentage composition					
	Moisture	Crude protein	Ether extract	Crude fiber	N-free extract	Ash
	(%)					
Experiment I, 1955–56						
Corn distillers dried grains [a]	8.4	26.4	7.2	11.5	41.8	4.6
Concentrate mixtures containing:						
Corn distillers dried grains	8.0	17.6	4.5	8.8	57.7	4.4
Brewers dried grain	8.4	18.8	3.5	9.5	65.4	4.4
Urea	9.6	19.3	3.7	4.2	59.6	3.8
No protein supplement	9.0	11.2	3.8	4.8	67.6	3.6
Mixed hay [b]	9.9	7.3	2.2	34.9	43.4	3.1
Grass silage [c]	66.8	4.2	1.2	9.8	16.1	1.9
Experiment II, 1956–57						
Concentrate mixtures containing:						
Corn distillers dried grains [a]	9.3	17.3	4.8	10.8	54.3	3.5
Brewers dried grains	9.1	18.1	4.5	10.2	53.8	4.3
Urea	9.9	18.2	3.5	5.3	59.4	3.7
No protein supplement	10.0	10.3	3.6	5.1	67.0	4.0
Timothy hay [d]	11.4	7.0	1.4	33.9	40.9	4.4
Corn silage [e]	78.4	2.2	0.6	6.5	11.2	1.1

[a] A blended sample furnished by the Distillers Feed Research Council, Cincinnati, Ohio, through the courtesy of Dr. L. E. Carpenter.

[b] U. S. No. 1 mixed hay consisting of 41.6% timothy, 20.6% quack grass, 18.4% red top and blue grass, 19.4% bird's-foot trefoil and vetch, and 1.1% foreign matter. The color index was 49%.

[c] Largely timothy and orchard grass, with a small amount of legumes, wilted before ensiling.

[d] U. S. No. 2 timothy hay, with 2.1% foreign matter. The color index was 37%.

[e] From well-eared corn, harvested in the late-dough stage.

ing of aliquots from four consecutive milkings. The cows were weighed on three days at the end of the preliminary, and at each 6-wk. experimental, period. The first week of each period was considered as transitional, and the data from the last 5 wk. were summarized for comparison. All data were analyzed by analysis of variance, and the Duncan *(3)* multiple range test was employed in evaluating treatment differences.

RESULTS AND DISCUSSION

The average daily milk and fat yields and the changes in body weights are summarized (Table 3). The data on significance for each individual experiment are included. It is clear that cows receiving the corn distillers dried grains and brewers dried grains produced more milk and more fat-corrected milk (FCM) than those on the low-protein diet. They also gained more weight. As an average, the results with corn distillers dried grains were superior to those with brewers dried grains.

On the low-protein concentrate, without a supplemental nitrogen source, milk production was poorest and the cows lost body weight in both experiments; whereas, they gained weight on the other feeds. These data clearly demonstrate that the basal, low-protein concentrate was too low in protein for the level of milk production obtained in these experiments, and it seems certain that if the experimental periods had been longer than the 6 wk. employed, milk production and body weights would have continued to decline at a faster rate than on the higher protein feeds. The addition of urea as the only nitrogen supplement overcame the downward trend. While, as an average, urea apparently was not as efficient as the nitrogen in either corn distillers dried grains or brewers dried grains for milk yield or weight gain, the differences were not statistically significant.

The fat percentage of the milk was slightly lower on the low-protein mixture in one experiment only.

TABLE 3

Average daily production of milk, fat, and fat-corrected milk and the changes in weight

Mixture containing	Average daily				
	Milk	Test	Fat	FCM	Weight gain
	(lb.)	*(%)*	*(lb.)*	*(lb.)*	*(lb.)*
	Experiment I, 1955–56				
Corn distillers dried grains	44.4[a]	3.64	1.62	42.0	0.75[a]
Brewers dried grains	44.3[a]	3.55	1.57	41.3	0.62[a]
Urea	43.6	3.60	1.57	40.9	0.18
No protein supplement	42.5	3.45[b]	1.46	39.0[b]	−0.10
	Experiment II, 1956–57				
Corn distillers dried grains	41.2[a]	3.58	1.48	38.6	0.61
Brewers dried grains	40.1[a]	3.59	1.44	37.6	0.94
Urea	39.6	3.59	1.42	37.2	0.43
No protein supplement	37.8	3.50	1.33	35.0[b]	−0.38[b]

[a] Significantly greater than the mixture containing no protein supplement ($P < 0.05$).
[b] Significantly less than the three other treatments ($P < 0.05$).

The concentrate mixture containing 3% of urea was less palatable than the other feeds. The cows consumed this mixture more slowly and with reluctance. Nearly half of the cows left small amounts of the urea-containing mixture for some hours after feeding, but this was usually consumed by the time of the next feeding and as it became mixed with the silage. Thus, the actual intakes of the different concentrates were approximately equal (Table 4). Other mixtures, containing 1.5–2.0% of urea and corn distillers dried grains, corn gluten feed, or soybean oil meal, to furnish the balance of the nitrogen in place of equal amounts of corn, were tested for palatability. All of those containing by-product feeds

TABLE 4

Average daily feed intakes and body weights

Mixture containing	Average daily intake of				
	Hay	Silage	Concentrate	Av. body weight	TDN/lb FCM
	—(lb.)—				
Experiment I, 1955–56					
Corn distillers dried grains	18.2	31.5	17.9	1376	0.56
Brewers dried grains	17.4	32.1	18.1	1384	0.57
Urea	18.8	31.1	17.1	1374	0.58
No protein supplement	17.2	32.3	18.1	1377	0.57
Experiment II, 1956–57					
Corn distillers dried grains	12.1	42.2	16.6	1254	0.57
Brewers dried grains	11.4	44.4	16.2	1234	0.58
Urea	11.9	43.2	16.2	1243	0.59
No protein supplement	12.2	45.5	16.1	1249	0.58

and having 2% or less of urea were eaten readily by the cows that showed reluctance to eat the high-urea feed. These observations show that although 3% of urea may be too high for satisfactory palatability in the kind of rations studied, up to 1.5–2.0% of urea can be used in usual mixtures with satisfactory results, both as to acceptability and as to milk yield.

The average daily intakes of feed and the average body weights of the cows are shown (Table 4). On the basis of Morrison's *(10)* values, it was estimated that the concentrate mixtures contained the following TDN values: distillers grains 74.9; brewers grains 68.1; urea 70.9, and low protein 73.3. Assuming that in Experiment I the mixed hay contained 47.8% TDN and the grass silage 21.1% TDN and, that in Experiment II the timothy hay contained 47.9% and the corn silage 18.3% TDN, the amounts of TDN required per pound of FCM produced were calculated as shown in Table 4. These calculations suggest that the mixtures containing corn distillers dried grains were somewhat more efficient for milk yield than the other concentrates, since less TDN was required per pound of FCM; however, these differences were not statistically significant.

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DETERMINATION OF THE INTRARUMINAL DISTRIBUTION OF SOLUBLE NITROGEN

W. E. C. MOORE AND K. W. KING

Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg

SUMMARY

An analytical fractionation procedure has been developed which facilitates relatively detailed nitrogen balance studies of the rumen fermentation. The procedure was evaluated in an in vivo experiment using three animals and three treatments. Regardless of dietary treatment, most of the nitrogen (65–78%) was observed to be found in feed residues and microbial cells. Only very small amounts of nitrogen (less than 2%) were encountered in the form of dissolved protein. Acceptable recoveries were obtained.

Previous efforts to elucidate the intraruminal metabolism of nitrogenous feed constituents have been based on analysis of one or two significant index compounds such as ammonia or a specific amino acid *(1–4, 6, 7, 9–11)*. Although such data are illuminating, they can not describe even over-all biochemical conversions as convincingly as do analyses allowing complete nitrogen balance.

The paucity of this type of information on rumen fermentation appears to arise from the fact that analytical procedures have not been adapted for determining the distribution of nitrogen among the several types of compounds known to be present in rumen ingesta. As a result of this analytical deficiency, balance studies have not been possible either in vivo or in vitro.

The present report consists of an evaluation of a micro-analytical procedure for analysis of the major nitrogenous components of rumen ingesta, using fistulated steers.

METHODS

The three fistulated steers used were 3-yr.-old Hereford-Guernsey stock fitted with plastic cannulas. During the pre-experimental period, they were maintained on a ration of good-quality timothy hay and approximately 4 lb. of 16% protein supplement daily. Water was available ad libitum.

On the day of the trials, duplicate samples of ingesta were taken from the top and bottom of the rumen of each animal at 8 A.M. and hourly thereafter until 3 P.M. Samples of bottom ingesta were obtained by opening the sample bottle after it had been lowered to the bottom of the organ immediately posterior to the anterior pillar of the rumen. All samples were immediately squeezed through four layers of cheese cloth before further processing.

For analysis of total nitrogen, a 1-ml. aliquot of the strained rumen liquid was diluted to 50 ml. with distilled water. Eight ml. of the strained liquid were centrifuged at 25,000 × G for 30 min. and 1.0 ml. of the supernatant was diluted to 10 ml. with distilled water for analysis of soluble nitrogen. Particulate nitrogen, consisting of feed particles and microbial cells, was calculated as the

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difference between the total and soluble nitrogen values. A further 1-ml. portion of the supernatant was diluted to 10 ml. with 11% trichloroacetic acid (TCA) and centrifuged at 25,000 × G for 10 min. Analysis of 1.0-ml. portions of this supernatant gave TCA-soluble nitrogen, and ammonia nitrogen was determined by direct nesslerization of a 1.0-ml. portion of the same supernatant. Soluble protein nitrogen was calculated as the difference between soluble nitrogen and TCA-soluble nitrogen. Organic nonprotein nitrogen was calculated as the difference between TCA-soluble nitrogen and ammonia nitrogen. All nitrogen analyses were made according to the micro-Kjeldahl procedure of Johnson *(8)*.

The animals were fed immediately after the 8 A.M. sampling. Treatment A consisted of hay fed ad libitum until 9 A.M. Treatment B consisted of 4 lb. of 16% protein grain supplement followed by hay ad libitum until 9 A.M. Treatment C consisted of 3 lb. of the same grain mixture plus 1 lb. of tryptone (Difco), followed by hay ad libitum for the remainder of the hour. Because tryptone was not palatable, it was mixed with the grain supplement and placed at the oesophageal orifice by reaching through the fistula. Three trials were run so that each steer received each treatment.

The analytical data were subjected to an analysis of variance in which the factors were tested against the appropriate interactions as shown in Table 1. The time-means of those nitrogen fractions which were found to vary significantly with time were grouped according to Duncan's multiple range test *(5)*.

RESULTS AND DISCUSSION

Organic nonprotein nitrogen, soluble nitrogen, and TCA-soluble nitrogen reached their highest observed average concentrations by 9 A.M., regardless of ration. From this time on, there was a consistent decrease in concentration until the next feeding. For each of these fractions the concentrations present after more than 4 hr. since feeding were indistinguishable from the prefeeding concentrations.

Ammonia, on the other hand, reached a maximum concentration 1 hr. after feeding stopped. Like the other nitrogenous fractions, however, ammonia concentrations were statistically significantly different from the prefeeding values only during the first 5 hr. after feeding.

As would be anticipated, total nitrogen values were maximal immediately after feeding. Within 3 hr. the concentration of total nitrogen was no longer distinguishable from the prefeeding values. An anomalously high value observed at 3 P.M. was not statistically significant.

Examination of the data (Figure 1) shows that the time-treatment interaction in organic nonprotein nitrogen may be the result either of the magnitude of the changes resulting from the dietary treatments or of the time at which maximum values occurred.

Table 2 summarizes all of the data describing the intraruminal distribution of nitrogen as a function of ration. Of special significance is the high percentage in feed residues and microbial cells. The concentrations of soluble protein were

TABLE 1

Statistical summary evaluating the effect of animal, treatment, time, and intramural sampling location on the distribution of nitrogen in the rumen

(Values are mean squares × 10^{-4})

Source	D.F.	Total-N	Particulate-N	Soluble-N	Soluble protein-N	Organic Non-protein-N	NH_3-N	TCA sol.-N
Animal	2	488.0	585.3	3.2	39.5	43.0	0.7	34.9
Treatment	2	95.2	456.4	243.8	6.5	252.4	7.9	348.0
Error (treatment × animal)	4	1,836.1	1,256.0	111.5	79.9	180.3	4.9	232.5
Time	7	470.4 [a]	145.9	131.1 [a]	25.2	200.7 [a]	3.4 [a]	239.5 [a]
Error (time × animal)	14	72.8	79.1	13.8	33.4	13.3	0.6	12.8
Time × treatment	14	81.8	30.5	25.7	26.6	72.0 [b]	0.5	70.9 [a]
Error (time × treatment × animal)	28	161.2	233.1	18.1	59.4	25.0	0.5	21.3
Location [c]	1	1,375.2 [b]	586.6	88.6 [b]	15.4	121.6 [b]	5.7 [b]	176.1 [b]
Error (location × animal)	2	49.7	48.6	1.3	2.0	4.8	0.2	6.4
Location × treatment	2	24.0	66.5	6.6 [b]	11.0	35.7	0.3	32.3
Error (location × time × ainmal)	4	38.6	28.6	.4	13.1	14.8	0.1	13.8
Location × time	7	24.1	32.5	7.7	4.5	17.7	0.05	17.9
Error (location × time × animal)	14	14.2	11.5	3.7	11.2	7.4	0.1	6.9
Location × treament × time	14	48.5	33.8	2.6	9.0	16.7	0.06	17.4
Error (location × treatment × time × animal)	28	53.6	77.6	4.9	16.8	8.8	0.08	8.8
Between duplicate sample error	144	25.1	30.1	4.8	11.3	5.7	0.02	5.7

D. F. = degrees of freedom.

[a] = significant at PL 0.01.

[b] = significant at PL 0.05.

[c] = location refers to top or bottom of the rumen.

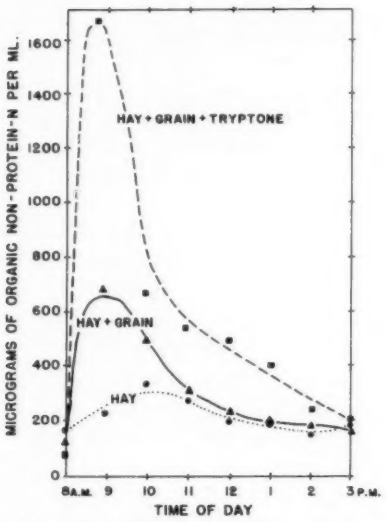

FIG. 1. Hourly fluctuations in ruminal concentrations of organic nonprotein nitrogen. (Points are average of top and bottom samples from three animals on three days.)

TABLE 2

Intramural distribution of nitrogen as affected by ration

Fraction	Ration					
	Hay		Grain		Grain + Tryptone	
	(γN/ml)	(% of total N)	(γN/ml)	(% of total N)	(γN/ml)	(% of total N)
I. Total-N	1,156	——	1,117	——	1,063	——
A. Particulate-N (I-A)	908	78.5	846	75.7	680	63.9
B. Soluble-N	229	19.8	269	23.0	387	36.4
1. TCA-soluble-N	212	18.3	257	21.9	395	37.1
2. NH_3-N	97	8.4	100	9.0	116	10.9
3. Soluble protein-N (B-1)	17	1.5	12	1.1	−8	−0.7
4. Organic NPN (1-2)	115	9.5	157	14.1	279	25.2
% Recovery [a]		97.9		99.9		98.4

[a] Calculated as $\frac{A + 2 + 3 + 4}{\text{total}} \times 100$.

generally so low on the dietary treatments used here that they fall within the range of analytical error. Of the fractions analyzed, organic nonprotein nitrogen was most sensitive to grain feeding. In general, the recoveries of nitrogen were well in excess of 95%.

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TECHNICAL NOTE

ASSOCIATION BETWEEN PLASMA AND LIVER CONCENTRATIONS OF VITAMIN A OR OF TOCOPHEROL IN HOLSTEIN MALE CALVES FED FIXED INTAKES OF THESE VITAMINS [1]

It is generally recognized that plasma vitamin A and plasma tocopherol concentrations increase in a diminishing manner with increases in intake of either of these vitamins, and that liver vitamin A and liver tocopherol concentrations increase in an essentially constant manner with increases in intake. This was especially apparent for those data in which animals were partially depleted of their body stores of these nutrients prior to supplementation with the vitamin(s), or in instances in which the supplementation periods were of sufficient duration to allow stabilization of the responses of both plasma and liver concentrations. From these relationships Almquist *(1, 2)*, in studying data from poultry, rats, lambs, and cattle, proposed that plasma vitamin A concentration was linearly related to the logarithm of liver vitamin A concentration. Although an initial study with calves *(8)*, over only a limited range of relatively low concentrations of plasma and liver vitamin A, indicated a linear regression between these two variables, a subsequent study *(10)* over considerably wider concentrations confirmed Almquist's finding of a linear regression between plasma and log liver vitamin A concentrations. Similarly, a pilot experiment with calves *(9)* gave evidence for a linear regression between plasma tocopherol and the logarithm of liver tocopherol. It was the purpose of this investigation to restudy the just-mentioned regressions in Holstein male calves over a relatively wider range of tissue concentrations of both vitamin A and tocopherol than heretofore available.

Data reported herein consisted of seven expreiments conducted at the Storrs Station during the period 1952–57. Immediately before being fed the fixed intakes of the vitamins, calves were fed an intake of a vitamin A depletion ration, also low in total tocopherol content, to provide a seven-day anticipated live weight increase of 10 lb. until partially depleted to plasma levels of either less than 12 γ of vitamin A or less than 100 γ of tocopherol per 100 ml. of plasma; or, the supplementation period was of sufficient duration to provide stabilization of the plasma levels. All animals were then fed—in addition to the vitamin A depletion ration—fixed intakes, adjusted at weekly intervals for each calf's individual live weight, of vitamin A per se from dry carriers, or its precursor, carotene, from artificially dehydrated alfalfa, and/or tocopherol from artificially dehydrated alfalfa or dry carriers, for periods ranging from 4 to 16 wk. duration. Vitamin A intakes ranged from 2 to 1,000 γ per pound live weight per day, carotene intakes from 12 to 540 γ, and tocopherol intakes from 0.1 to 25.0 mg. The plasma and liver concentrations represent those values, at the termination of each animal's supplementation period, at which time the average live weight was 406 lb., with a standard deviation per animal of 96 lb. (range 152–590 lb.). Plasma vitamin A concentrations were determined by the Kimble procedure *(6)*, liver vitamin A by the Davies or modified Gallup-Hoefer procedures *(4)*, and plasma tocopherol and liver tocopherol as previously described *(9)*. Statistical procedures used were those as outlined by Anderson and Bancroft *(3)* and Quenouille *(7)*. Because of the relatively few animals within any one experiment, no attempt was made to present the regressions between plasma and liver by individual experiments. However, when studied individually, good agreement existed between experiments, especially when consideration was given to the range of concentrations represented by any one experiment as contrasted with another.

In Table 1, the plasma concentrations of either vitamin A or tocopherol, according to 0.400 log intervals of liver concentrations of vitamin A or tocopherol, are presented. As can be seen (by reference to Columns 4 and 5 of Table 1), the variances of the plasma concentrations increased approximately proportionally to the square of the mean plasma concentration (or, the coefficient of variation of plasma concentration did not change appreciably with an increase in the mean from which it was derived). Therefore, in derivation of linear regression of plasma concentrations on log liver concentrations, weighting the means of both variables by the reciprocal of the square of its mean plasma concentration was used to meet the requirements of constant variance for analysis of variance, application of tests of significance, and derivation of errors about the regression. These regressions (Table 2) appear

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TABLE 1

Relationships of plasma vitamin A to log liver vitamin A and plasma tocopherol to log liver tocopherol

No. of calves	Log (liver vitamin A in γ/100 g.), X_1		Plasma vitamin A in γ/100 ml., Y_1	
	Range	Mean	Mean	Variance
6	0.600–0.999	0.911	6.1	3.60
11	1.000–1.399	1.208	7.6	6.04
13	1.400–1.799	1.637	9.2	6.37
18	1.800–2.199	2.061	13.5	15.78
18	2.200–2.599	2.448	18.2	12.06
21	2.600–2.999	2.795	22.6	31.90
16	3.000–3.399	3.214	26.7	16.50
9	3.400–3.799	3.626	33.8	11.79
4	3.800–4.199	3.897	32.0	40.47
0	4.200–4.599	.....	.....	.....
9	4.600–4.999	4.860	52.0	56.82
No. of calves	Log (liver tocopherol in γ/100 g.), X_2		Plasma tocopherol in γ/100 ml., Y_2	
	Range	Mean	Mean	Variance
7	1.600–1.999	1.856	64	127
32	2.000–2.399	2.293	133	1,656
39	2.400–2.799	2.547	192	1,657
10	2.800–3.199	3.055	276	12,583
11	3.200–3.599	3.446	461	18,745
0	3.600–3.999	.....	.....	.....
3	4.000–4.399	4.060	784	12,462

TABLE 2

Linear regressions of plasma concentration and log liver concentration of vitamin A and of tocopherol

	Coefficients of the linear regressions		Standard error of estimate	Simple correlation coefficient
	Intercept	Slope		
	(a)	*(b)*	$(s_{y \cdot x})$	(r)[d]
Linear regression of plasma concentration in γ/100 ml. on log (liver concentration in γ/100 g.)[a]				
Vitamin A	− 3.7 ± 1.3[c]	9.08 ± 0.80[c]	4.87	0.97
Tocopherol	−311 ± 52	197.88 ± 23.20	53.66	0.97
Linear regression of log (liver concentration in γ/100 g.) on plasma concentration in γ/100 ml.[b]				
Vitamin A	1.018 ± 0.144	0.0750 ± 0.0025	0.36	0.94
Tocopherol	1.998 ± 0.470	0.0028 ± 0.0007	1.09	0.90

[a] Weighted regressions of the means (Table 1) in which both variables were weighted by a factor $10^2 n/\bar{y}_1^2$ in the case of vitamin A and by a factor of $10^4 n/\bar{y}_2^2$ in the case of tocopherol, when n = number of calves per group and $\bar{y}^2$ = the square of the mean plasma concentration for the particular group.

[b] Regressions derived from individual calf data.

[c] Standard error.

[d] The differences in the correlation coefficients, between the linear regressions of plasma concentration on log liver concentration and of log liver concentration on plasma concentration, were due to using grouped and weighted data in the first case and individual calf data in the second.

to confirm Almquist's *(1, 2)* previous studies, in that there exists a linear regression of plasma vitamin A concentration on log liver vitamin A concentration. Also, a linear regression of plasma tocopherol concentration on log liver tocopherol was found. Due to the high correlation coefficients, and the considerable labor involved in deriving second-order equations when weighting is employed, the possible existence of curvature in these relationships was not studied.

Of possible greater importance than the above linear regressions, was the prediction of liver concentration of vitamin A or of tocopherol from plasma concentration. Since neither of these variables can be considered dependent upon the other under conditions of fixed intake of the respective vitamin, it was, therefore, necessary to derive regressions of log liver concentration on plasma concentration from which liver concentrations of vitamin A or tocopherol can be estimated from the concentration of vitamin A or tocopherol in calf plasma. Such linear regressions are given in the last two lines of Table 2. When log liver concentrations were grouped using 5.0 γ plasma vitamin A per 100 ml. as the interval size over the range 0.0–64.9 γ, and using 75 γ plasma tocopherol per 100 ml. as the interval size over the range 0–899 γ, no systematic changes were observed in the variances of log liver concentration. Therefore, no weighting was indicated and the regressions were derived based on individual calf plasma and log liver concentrations. These linear regressions had applicable limits for estimation of log liver vitamin A in γ/100 g. from 3.8 to 64.8 γ of plasma vitamin A and for log liver tocopherol in γ/100 g. from 45 to 899 γ of plasma tocopherol. Standard errors of log liver concentration predicted from a particular value of plasma concentration were for log liver vitamin A concentration:

$$\sqrt{0.127{,}890\left\{1+\frac{1}{125}+\frac{(Y_1-20.9)^2}{19{,}671.61}\right\}},$$

and for log liver tocopherol concentration:

$$\sqrt{1.188{,}984\left\{1+\frac{1}{102}+\frac{(Y_2-219)^2}{2{,}510{,}920}\right\}},$$

in which Y_1 and Y_2 are, respectively, plasma vitamin A and plasma tocopherol concentrations from which log liver concentration is predicted.

For adequate interpretation of these linear regressions, it needs to be recognized that the variables used in their derivation were not selected strictly at random, since the intakes of the respective vitamins were fixed [see Davies *(5)*]. Thus, because of this, plasma and liver concentrations were limited in their respective distributions. However, no selection was made of the plasma or liver concentrations and these criteria, of course, varied about a particular intake. Therefore, in the latter case, plasma and liver concentrations can be regarded as random. Because of these restrictions, and the existence of measurement errors in the determination of of both plasma and liver concentrations of either vitamin, estimates of the functional relationship between plasma and liver are not feasible. Therefore, it is necessary to limit the use of the linear regressions to predictions under conditions of fixed intake of the respective vitamins, and to evaluation of the association between the two variables, plasma and liver. In addition to these considerations, it is recognized that under certain dietary regimes *(11)* plasma and liver concentrations may be changed markedly in relation to each other. Thus, in the use of these regressions for prediction purposes, the constituents of the ration fed to the calf need to be allowed for.

H. D. Eaton
J. E. Rousseau, Jr.[2]
Martha W. Dicks
R. Teichman[3]
A. P. Grifo, Jr.
Storrs (Conn.) Agricultural Experiment Station

[2] Present address: U. S. Fishery Products Lab., Ketchikan, Alaska.

[3] Present address: Animal Industry and Experimental Statistics Dept., North Carolina State College, Raleigh.

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OUR INDUSTRY TODAY

TREATMENT OF MILK FOR CHEESE WITH HYDROGEN PEROXIDE[1,2]

Z. D. Roundy

Food Research Department, Armour and Company, Chicago, Illinois

The ripening of cheese is due to enzymes secreted in the milk, to the rennet extract added to coagulate the milk, and to microorganisms growing symbiotically and in proper sequence in the cheese. Mixed with the beneficial bacteria of most milk supplies will be found harmful types. If these are abundant, or find conditions favorable for growth, they are certain to have an undesirable influence on the ripening of the cheese. Control of the bacteria in the manufacture of cheese is, therefore, of paramount importance.

Beginning about 1890, notable advances were made toward the control of the fermentation process in cheese. The principal ones were: *(1)* the use of specially prepared cultures, *(2)* ripening of the cheese at cooler temperatures, and *(3)* pasteurization of the milk. Pasteurization of milk for cheese has been advocated by public health officials because it destroys pathogenic organisms that may be present and, by industry, because it destroys certain types of organisms harmful to cheese-making, including the pathogens. While pasteurized milk generally permits better end-results than raw milk, there are some objections to pasteurization. It inactivates important natural enzymes in the milk and destroys beneficial organisms. As a result, cheese made from pasteurized milk ripens slowly and is milder in flavor than cheese made from raw milk.

Research efforts of the cheese industry have long been directed toward further improvements in the quality and uniformity of cheese, and means by which the ripening process can be accelerated. Pursuant to these objectives, a number of methods have been tried, but without marked success, such as adding to milk enzymes from a variety of sources, applying heat treatments slightly lower than pasteurizing temperatures, and using mixtures of pasteurized milk and raw milk.

Hydrogen peroxide, which was discovered in 1818 by the French chemist L. J. Thenard *(21)* has, for many years, been recognized as a bactericidal agent.

In 1883, Schrodt *(19)*, in Germany, published on the preservation of milk by hydrogen peroxide. In 1900, Low *(7)*, of the U. S. Department of Agriculture, is said to have used peroxide to preserve milk. In France, Jablin and Gonnet *(6)* in 1901, Renard *(18)* in 1904, and Nicolle and Duclaux *(16)* in 1904, were also interested in preserving milk with peroxide.

The Danish Engineer Budde *(2)*, in 1904, was presumably the first to attempt to produce sterile milk with a combination of heat (51° C.) and hydrogen peroxide. He depended on the peroxidase and catalase naturally present in the milk to decompose the peroxide. The milk produced by the heat-peroxide process was known as "Buddized" milk.

In 1906, Much and Römer *(14)*, in Germany, studied the use of hydrogen peroxide and a catalase-containing material, blood serum, as a means of sterilizing milk for children over 6 yr. of age. Prior to that time, however, Senter had prepared an enzyme material (Hämase) from blood, which he found would decompose hydrogen peroxide.

From about 1906 to the present time, many papers have appeared that relate to the preservation of milk by hydrogen peroxide and to the use of such milk, in some instances, in the manufacture of cheese. The French, Germans, and Italians have been especially active in this field. Unfortunately, the results pertaining to the manufacture of cheese from peroxide-treated milk generally have been disappointing.

Arnaudi and Trecani, in 1953 *(1)*, studied the influence of the hydrogen peroxide treatment on the rate of coagulation of milk and firmness of the curd. They found that the addition of 0.5 to one part of 35% (130-vol.) hydrogen peroxide per thousand improved the coagulation time. The addition of two parts or more per thousand delayed coagulation and decreased the firmness of the curd.

Peltola and Mattson, in 1950 *(17)*, in Finland, had difficulty in obtaining dry cheese from milk preserved with 1% hydrogen peroxide.

Morris, Larsen, Johnson, in 1951 *(13)*, in this country, experimented with Swiss cheese made from raw milk, pasteurized milk (phosphatase-negative), and hydrogen peroxide–catalase treated milk. The milk was treated at 120° F. for 30 min. with 0.2% of a 35% solution of hydrogen peroxide. The results obtained were of interest. Eighty per cent of the treated cheese was Grade A, while only 20% of the raw-milk cheese and none of the pasteurized-milk cheese was Grade A.

Morris, in 1951 *(12)*, also made Cheddar

[1] Presented at Research Conference at Beltsville, Maryland, October 30, 1957, held by the Eastern Utilization Research and Development Division, USDA, Philadelphia, Pa.

[2] A paper on this subject appeared recently by L. J. Tepley, P. H. Derse, and W. V. Price. *J. Dairy Sci.*, **41**: 593. 1958.

cheese from raw milk, pasteurized milk, and hydrogen peroxide–catalase treated milk. He found that the cheese from the treated milk was higher in moisture than the other cheeses, and that the high-moisture cheeses had a pasty texture and soft body. Bitterness and high-acid flavors also were common defects.

Lück and Joubert *(9)* have done some of the most recent and extensive work on milk preserved with peroxide. Articles by one or both of them have appeared in *Milchwissenschaft*, the *Dairy Science Abstracts*,[3] and other publications.

Lück *(8)* used milk preserved for 24 hr. with 0.25% of a 35% (130-vol.) solution of hydrogen peroxide, in the manufacture of Cheddar and Gouda cheeses. Since these cheeses contained body and flavor defects, he did not recommend the process. According to Lück, the addition to milk of a smaller amount of peroxide, at more frequent intervals, gave better results than could be obtained by adding a large amount of peroxide at one time. The quality of cheese also improved when treated milk was mixed with untreated milk.

Heidrich *(5)* reported that Emmental and Tilsit cheeses made from milk treated with 0.2% of a 30% solution of hydrogen peroxide (chemically pure Perhydrol) for 30 min. at 50–55° C. were good, but no better than those obtained from pasteurized milk.

The effect of peroxide on enzymes has been studied by several investigators. Waksman and Davison *(22)* stated that catalase is sensitive to peroxide but that pepsin and trypsin are not. Cimino, in 1945 *(3)*, found that hydrogen peroxide (0.2–0.3% electrolytic 39% H_2O_2 solution) added to cow's milk both at freezing and at 20–30° C. did not affect amylase, lipase, tryptase, and phosphatase, but nearly destroyed peroxidase, catalase, and reductase.

Our work on the hydrogen peroxide–catalase treatment of milk to be used in the manufacture of cheese dates back to the 1940's. The result of this work is a process which provides a selective destruction of most of the undesirable bacteria in milk, without an adverse effect on the milk itself and the acid-forming organisms in it.

Hydrogen peroxide is unique among germicides. It can be used to destroy organisms in milk and, after it has done its work, it can be rapidly and completely broken down into oxygen and water. Furthermore, hydrogen peroxide as it is produced today is remarkably stable and, when used in conjunction with catalase, does not impart an off-flavor to the milk.

For more than 50 yr., hydrogen peroxide has been used from time to time in milk, but during part of this time there was no good method available to decompose the peroxide. The object of its use primarily was to extend the keeping quality of milk for a period of one or more days by preventing bacterial increase.

In 1937, Sumner and Dounce *(20)* announced the preparation of crystalline catalase from beef liver, which was relatively stable and very active. Catalase is now regarded as one of the most effective enzymes known. It has been calculated that one molecule of catalase (mol. wt., 225,000) can in 1 min. decompose 2,600,000 molecules of hydrogen peroxide at 0° C.

Catalase is found in high concentrations in blood, liver, kidneys, and fatty tissues. The catalase preparations used in this work were made by The Armour Laboratories from beef liver. They were designed to be suitable for use in food processing; active, stable, and uniform from batch to batch and from day to day; tasteless; if made in powdered form, readily soluble or dispersible; free or relatively free from microorganisms, and reasonable in price.

The first catalase preparation used in this work was in powdered form and was standardized to a potency of 3,000 Keil Units per gram. One Keil Unit is defined as the quantity of catalase required to decompose 1 g. of hydrogen peroxide in 10 min. at 25° C., in an inert atmosphere of CO_2 or N_2. The present product, Armalase A-100, which for convenience is now generally used in place of the powdered material, is an amorphous preparation in aqueous solution. It has been standardized to a potency of 100 Keil Units per milliliter.

Studies on the peroxide treatment of milk for cheese were directed along the following lines: chemical, bacteriological, methodological, and nutritional. The present discussion is confined to the method of treating the milk, the effect of peroxide on certain types of organisms in milk, and the quality of the cheese.

A method for treating the milk. The development of a practical method for treating the milk with peroxide was given primary consideration. Two methods were investigated: the vat method and the so-called flash method. In the former, the milk was treated in the vat in which the cheese was made, at temperatures either between 86 and 94° F. (setting temperatures) or between 120 and 130° F. The amount of peroxide used in these first experiments was 0.06% (ased on 100% peroxide), with periods of exposure of 20–30 min. Catalase was then added to decompose the peroxide.

Treatment of milk at the setting temperature gave fairly satisfactory results, yet there were disadvantages connected with this procedure. First, the catalase naturally present in the milk decomposed some of the peroxide before it had time to act on the organisms. Secondly, peroxide is less active at the setting temperature than at higher temperatures. The activity of hydrogen peroxide solutions is said to increase approximately two times for each 10° C. rise in temperature, other factors being equal.

Vat-treatment of milk at 120–130° F. inactivated most of the indigenous catalase and increased the activity of the peroxide. However, treatment of milk in the vat at temperatures

much in excess of the setting temperature was considered impractical for large-scale operations, because heating the milk is a slow process and adequate cooling facilities are not always available.[3] Milk treated at 120–130° F. for 20–30 min. with 0.06% peroxide, then with catalase to decompose the peroxide, gave variable results. In some instances, the cheese was of satisfactory quality, but often it had a high moisture content and soft body. Bitterness and an acid flavor often developed in the high-moisture cheese during ripening. These defects were attributed to over-treatment of the milk. As the work progressed, the concentration of peroxide and period of exposure were reduced, with improved results.

The flash method was investigated next. In preliminary experiments with the flash method, the milk was treated at 120–130° F. for 20–40 sec. with 0.06% peroxide, then with catalase to decompose the peroxide. Although this treatment produced cheese of better quality than could be obtained from milk treated at 120–130° F. for 20–30 min. with 0.06% peroxide, it destroyed most of the acid-forming organisms. The concentration of peroxide was then reduced to permit large numbers of the acid-forming organisms to survive. Milk treated at 120–130° F. was of very good quality. For example, treatment of milk at 125° F. for about 25 sec. with 0.02% peroxide destroyed nearly all the gas-forming organisms and left intact more of the acid-forming organisms than are left by accepted pasteurization procedures. The cheese made from such milk ripened faster and usually had a finer body and flavor than cheese made from pasteurized milk. Treatment of milk by the flash method, with a reduced amount of peroxide, hastened the cheese-making process, reduced the cost, minimized foaming, and usually improved the quality of the cheese. Incidentally, the enzyme phosphatase was not destroyed by the flash treatments used. A brief description of the flash method finally adopted for routine experimental purposes is as follows:

Clarified milk is treated in the surge tank with 0.02% hydrogen peroxide (about 0.06% of a 35% solution of hydrogen peroxide, designed for use in food processing, diluted with an equal weight of cold water). The peroxide solution is added to the milk by means of a flowmeter. From the surge tank, the treated milk passes through a high-temperature, short-time (HTST) plate-type pasteurizer, where it is heated to approximately 125° F. for about 25 sec. It is then cooled to the setting temperature (86° F. for Cheddar cheese or 94° F. for Swiss cheese) before it is delivered to the vat. During filling, or when the vat is full, catalase is added to decompose the peroxide. It is essential that all the peroxide in the milk be decomposed before starting the cheese-making operations; otherwise, the starter organisms fail to grow. Excess catalase does no harm. Three to four times the required theoretical amount of catalase, diluted with about five times its volume of cold water, give good results.

The milk is tested for the presence of hydrogen peroxide by adding a few drops of a fresh 25–30% solution of potassium iodide with 2% soluble starch to about 5 ml. of milk in a tube. A control tube of identical milk not treated with peroxide is prepared in the same manner. Identical colors in the two tubes indicate destruction of the peroxide. A 2% solution of *para*-phenylenediamine dihydrochloride, or a 25–30% solution of potassium iodide (without starch), also has been used successfully for testing milk for the presence of peroxide. The test with these reagents is carried out in the same manner as described with potassium iodide and starch. As soon as the milk is free of peroxide, starter is added and the cheese is made in the usual manner. Needless to say, only stainless steel or equally corrosion-resistant material should be used in connection with the peroxide method.

Effect of hydrogen peroxide on microorganisms. It has been shown that among the bacteria that failed to produce catalase were the anaerobes, and these are very sensitive to hydrogen peroxide *(10, 11)*.

In 1940, Curran, Evans, and Levitan *(4)* studied the sporicidal action of hydrogen peroxide. They found that pH, concentration, number of exposed organisms, and temperature were important factors in connection with sporicidal activity.

Nambudripad and coworkers, in 1949 *(15)*, exposed coliform organisms to different concentrations of hydrogen peroxide at 37° C. for various lengths of time and found that none of the *Escherichia coli* exposed to 500 p.p.m. (0.05%) for 10 min. were able to survive. Only 6.77% of them survived treatment with 300 p.p.m. (0.03%) peroxide for 30 min. None of the *Aerobacter aerogenes* organisms exposed to 500 p.p.m. for 30 min. survived. Only 55.89% of them survived a peroxide treatment of 300 p.p.m. for 30 min. The *Streptococcus lactis* and *Lactobacillus bulgaricus* organisms were considerably more resistant to destruction by peroxide than the coliform organisms.

In this work, bacteriological analyses were made[4] to determine the effect of peroxide on representative species of microorganisms. Briefly, the procedure used to evaluate the peroxide treatment was as follows: To 150 ml. of sterile milk in a 500-ml., triple-necked distillation flask, equipped with a motor-driven stirring device, was added 2 ml. of a 24-hr. broth culture

[3] Vat-treatment of milk under conditions that obtain in the pilot-plant was carried out without difficulty.

[4] I am indebted to Dr. L. A. Harriman and coworkers (Food Research Department, Armour and Company) for the bacteriological data.

of the test organisms, followed as quickly as possible by the designated amount of peroxide. The temperature was maintained for the desired time, after which the flasks were cooled to 95° F. in ice water. Catalase[5] was added to decompose the peroxide. Standard plate counts were then made to determine the number of organisms surviving the treatment. Table 1 shows the effect of peroxide on certain lactic acid organisms and the propionic acid organisms, *P. shermanii*. It will be noted that nearly all of the *S. lactis, L. bulgaricus,* and *P. shermanii* organisms were destroyed by the treatment used. In view of this fact, the 0.06% peroxide, which had been used to treat the milk for cheese, was gradually reduced to 0.01–0.03% to permit as many as possible of the acid-forming organisms to survive.

Table 2 shows the effect of peroxide on organisms often associated with defects in cheese and other dairy products.

Since spores of *B. subtilis* and *C. butyricum* were unaffected by 0.06% peroxide (as shown in Table 2), the effect of peroxide on spores was further investigated. Table 3 shows the percentage reduction of spores exposed to different amounts of hydrogen peroxide for different lengths of time. Unfortunately, these periods of exposure are not all comparable for the two organisms.

[5] The catalase preparations used in these experiments contributed from zero, in most instances, to five organisms per milliliter of milk.

TABLE 1

Reduction of microorganisms exposed to 130° F. for 1 min. with and without hydrogen peroxide

	Peroxide (%)	
	0	0.06
	(%)	(%)
Streptococcus lactis	34.5	99.9
Propionibacterium shermanii	52.8	99.9
Lactobacillus bulgaricus	32.7	98.0
Streptococcus thermophilus	12.0	29.0
Streptococcus diacetilactis (L62)	21.0	45.0
Streptococcus diacetilactis (L63)	0.0	0.0
Streptococcus diacetilactis (L64)	8.0	5.0

TABLE 2

Reduction of microorganisms exposed to 130° F. for 1 min. with and without hydrogen peroxide

	Peroxide (%)	
	0	0.6
	(%)	(%)
Escherichia coli	6.9	99.9
Aerobacter aerogenes	94.2	99.9
Pseudomonas fragii	82.1	82.6
Pseudomonas fluorescens	88.5	99.9
Bacillus mesentericus[a]	92.6	99.7
Clostridium perfringens[a]	36.5	99.9
Clostridium butyricum[a]	92.6	99.8
Bacillus subtilis[a]	90.5	99.9
Torula cremoris	50.6	52.5
Torula sphaerica	99.9	99.9
Bacillus subtilis spores	9.2	0.0
Clostridium butyricum spores	0.0	0.0

[a] Mostly vegetative cells.

TABLE 3

Reduction of spores exposed to different amounts of hydrogen peroxide

Culture	Temp.	Time	Peroxide (%) 0	0.06	0.30
	(°F.)	(min.)	———	(%)	———
B. subtilis	130	1	9.2	0.0	29.3
B. subtilis	140	1	14.6	....	33.0
B. subtilis	140	10	36.6	....	63.7
B. subtilis	140	30	7.3	....	99.5
C. butyricum	130	1	0.0	0.0	57.8
C. butyricum	130	5	0.0	....	83.3
C. butyricum	130	10	0.0	....	.....
C. butyricum	140	30	9.7	99.8	99.9

TABLE 4

Reduction of microorganisms in milk treated at a cheese factory at 127° F. for 40 sec. with hydrogen peroxide

Peroxide (%)	Reduction (%)		
	Number of trials	Total bacteria (average)	Coliform (average)
0.06	8	84.04	99.99
0.047	7	82.35	99.99
0.038	3	78.67	99.99

The data in Table 4 were obtained at a cheese factory. The milk was treated in a surge tank with various amounts of peroxide, as indicated. The treated milk was then passed through a plate-type flash pasteurizer, where it was heated at 127° F. for 40 sec.

The bacteria counts (not shown in the table) on milk of similar quality that had been pasteurized at approximately 163° F. for 40 sec. showed that 99.99% of the coliform bacteria and 99.90% of the total bacteria were destroyed.

Table 5 shows the results obtained (under laboratory conditions) using the same heat treatments as just described, but smaller amounts of peroxide. The primary object of the test was to determine whether these smaller amounts of peroxide would destroy the coliform organisms.

It was of interest to observe (data not given in the table) that a substantial number of different types of acid-forming organisms were able to survive the above treatments.

Table 6 shows the effect of peroxide on certain pathogenic organisms added to milk.

DISCUSSION AND COMMENTS

This discussion has been confined to the use of hydrogen peroxide and catalase in the manufacture of Cheddar and Swiss cheeses. How-

TABLE 5

Reduction of microorganisms in milk treated at 127° F. for 40 sec. with different amounts of hydrogen peroxide

Peroxide (%)	Reduction (%)	
	Total bacteria	Coliform
0.13	82.17	99.99
0.015	65.00	99.99
0.008	57.17	99.99

TABLE 6

Reduction of pathogenic microorganisms exposed to 130° F. for 1 min. with and without hydrogen peroxide

	Peroxide (%)	
	0	0.06
	(%)	(%)
Micrococcus pyogenes	8.8	97.8
Salmonella enteriditis	96.5	99.99
Salmonella tennessee	76.4	99.92
Salmonella pullorum	98.9	99.99
Salmonella typhimurium	99.3	99.99
Shigella paradystenteriae	0.0	99.96

ever, other types of cheese have been successfully produced by this method.

Swiss and Cheddar cheeses of excellent quality and uniformity have been produced from milk treated with hydrogen peroxide and catalase. Hydrogen peroxide has a selective action on bacteria. The aerobic, spore-forming organisms are most resistant to destruction by peroxide and the coliform organisms the least resistant. The susceptibility of the lactic acid organisms is intermediate. The success of the peroxide-catalase treatment of milk for cheese depends, to a great extent, on selecting conditions of treatment that will destroy the undesirable organisms and permit the desirable ones to survive. Actually, the amount of peroxide needed will depend on the bacteriological quality of the milk, the temperature of the milk at the time the peroxide is added, and the duration of treatment.

It has been shown by curd-tension measurements that hydrogen peroxide softens milk proteins slightly. Whether this softening is due to an unfolding of the protein molecule or to some other reaction is not clear. While mild peroxide treatments of milk had a desirable effect on the body of the cheese, high concentrations and long exposures to peroxide had the opposite effect. The former produced cheese with a medium-firm and elastic body. The latter produced cheese with a relatively high moisture content and soft body. As indicated, bitterness and an acid flavor often developed in the high-moisture cheese as it aged. Oxidized or "cappy" flavors did not develop in milk treated with hydrogen peroxide and catalase.

The benefits derived from the use of the peroxide-catalase method are believed to be due to: *(1)* the selective action of peroxide on bacteria, in which organisms causing common defects in cheese are largely destroyed and many desirable organisms are able to survive; *(2)* activity of natural enzymes in milk (and possibly catalase added to the milk), and *(3)* the effect of peroxide on milk proteins, which results in the production of cheese with a medium-firm, elastic body.

The principal disadvantages of the hydrogen peroxide–catalase method are: *(1)* The time required to make the cheese is slightly longer than by conventional methods (the peroxide must be destroyed before the starter is added). *(2)* The cost of manufacture by the peroxide method is slightly higher than by conventional methods (the peroxide and catalase usually cost between 0.1 and 0.5¢ per pound of cheese).

The peroxide-catalase method of treating milk for cheese is easily applied and, based on the results of these studies, has good possibilities for improving cheese quality. It would be a mistake, however, to expect the process to cure everything. Milk which contains large numbers of undesirable organisms is quite certain to produce cheese of inferior quality. We should not relax our efforts to produce good, clean milk, and we should make every effort to improve it.

While the antiseptic and germicidal properties of hydrogen peroxide are well known, the application of the method to cheese-making is relatively new and merits further investigation.

To make cheese by the hydrogen peroxide–catalase method, one should obtain permission from regulatory officials.

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REMINISCENCES ON DAIRY CATTLE BREEDING

M. H. Fohrman

Animal Husbandry Research Division, USDA, Beltsville, Maryland

How many of you readers recall the "rag doll" germination test for seed corn? Those who do also remember seeing the carefully selected ears of corn hanging from the rafters of the barn or feed room, to protect them from damage by rats and mold. These are memories of the days when most farmers selected their seed corn in the field and, in a sense, were corn breeders. The introduction of hybrid corn has made corn breeding and seed corn production a highly specialized business, and the modern farmer has benefited tremendously from this revolutionary change in producing this basic crop.

Maybe some of you can recall when the barnyard flock of chickens was replenished by random breeding and nest incubation. Nowadays, even if the small flock of hens is maintained only to supply the home needs, it is quite apt to be replaced yearly with a cardboard box of chicks from a commercial hatchery. These are bred-to-order for the customer.

Now, when we turn to the dairy herd the story changes, as no one has yet established a business of supplying satisfactory replacements in sufficient quantity to meet the needs of our dairy industry. The significant difference here is that the dairy farmer must still be a breeder and produce his future herd from the animals he now has. If we consider the commercial dairy farm as a milk manufacturing business, then the barn becomes the factory and the cows are the machines which are housed in the factory to produce milk from the raw materials which the dairyman supplies. His first concern should be to see that each space in that factory is occupied by a modern and efficient machine. Next, he should be sure that he is supplying good raw material in sufficient quantity to insure optimum use of his milk machines.

When we continue the parallel, we find that the successful factory owner is constantly alert to improvements in the machines he uses, but the job of making better machines is mostly taken care of by specialists whose business it is to develop new machinery. Here the parallel ends, as the owner of the milk factory is, to a large extent, responsible for initiating and growing the new machines which are to replace those he is now using. This is a point which should be given quite a bit of serious consideration by all who are interested in the betterment of our dairy industry. Some time ago I attended a meeting of young dairy farmers, many of whom had a background of 4-H and Future Farmer training. This was a discussion group, all interested in becoming better farmers. What surprised me most was the lack of basic knowledge of the part of their business which had to do with proper breeding procedures to follow, in order to insure some progress in the producing ability of their future herds. It was quite evident that the majority of these young dairymen were more concerned with how the new machines would look than with how much milk they would produce. No doubt this was due to overemphasis on type during their training periods, and wishful thinking that if their heifers looked good they would produce well.

What approach can be suggested to overcome this deficiency? Most college courses are too technical to be of much help to operating dairymen. Many popular articles on dairy cattle breeding are postulated on the assumption that like begets like, which makes them overly simple, with undue emphasis on line-breeding. Research reports based largely on records from herds not under experimental control are heavily larded with statistics, which put the results beyond the reach of dairy farmers and breeders. It should be said here that the fault is largely due to lack of funds at our research institutions, to support herds of sufficient size to produce adequate data for study. Teaching and training of leaders is always more effectively done when it is supported by live research to supply the material and stimulate the interest in problems involved in breeding better dairy cattle. This is not criticism or apology, but only a statement of existing conditions. During the past 10 yr. there has been a positive movement to expand the research in this field, and the condition should rapidly improve.

In the meantime, what can be done to afford some guidance to our dairymen who are interested in improving their herds by breeding? First of all, the use of artificial breeding should be encouraged and recommended. Improvement in producing ability can be accomplished by centering the breeding effort on good production-proved bulls. Thinking along the lines of improvement should be based on the existing condition, which is, that only a small percentage of our producing dairy cattle is pedigreed. There is no line of cleavage between registered and nonregistered cattle in the application of sound breeding principles.

Realizing that the future worth of any man's herd depends so largely upon the quality of the sires he uses, the recommendation of artificial breeding is sound. The production-proved sire carries us beyond the speculative operation of pedigree analysis. Pedigree selection was the main reliance of breeders for many, many years, but its over-all effects are not too impressive. Production-proved sires at least afford a preview of their future performance. There is a tendency now to overanalyze existing data and to use mathematical adjustments as a substitute for what might be called "cow sense". We might state that once this matter of bull selection becomes an abstract mathematical exercise it loses its appeal to the man who is perhaps best endowed to make a success of operating a breeding establishment. It appears now that a combination of skills will bring the best results in assessing the real value of service sires.

Despite the periodic misgivings which crop up regarding the value of proved sires, the fact remains that most bull studs still promote their activities on the basis of selling service to bulls of this kind, and there is reason to believe that this is sound and will continue, as no adequate substitute is as yet in sight.

The Holstein herd at Beltsville affords the best proof of the effectiveness of proved-sire breeding in developing a herd to a uniformly high level of milk production. Seven unrelated sires were used in sequence and the program has been continued through the use of semen from production-proved sires in artificial breeding studs in Pennsylvania and New York. The foundation herd of 17 females averaged 542 lb. of butterfat. When analyzed on a generation basis, the results show that there is a steady increase in the production level of each generation over that of the preceding one, and 23 cows in the eighth generation averaged 720 lb. of butterfat. The increases average about 14 lb. of fat per generation, and this same figure is arrived at by a comparison of dams and daughters for the entire herd. Almost 400 females have completed production records on this project and about 100 of these were sired by bulls that were in service in the artificial-breeding studs. Variability showed a marked decline after the third proved-sire generation.

Having offered an acceptable and workable plan of breeding on the male side, it might be well to consider what benefits can be added by female selection, an option under control of the dairyman or breeder. The Beltsville breeding experiment was conducted without selection on the female side and, for this reason, the complete data afford a basis for determining what, if any, benefit will follow if female selec-

tion is practiced. Since the advent of cow testing, dairy farmers have been advised not to raise replacements from their lower-producing cows. This was based on the assumption that like begets like, and the practice itself defeats any effort to assess its effectiveness. A study of the unselected data from the Beltsville herd has yielded some rather interesting results. The foundation group included four cows with records below 425 lb. of butterfat. They had no immediate progeny which fell below this modest culling level. Of the 16 cows in the bred herd which made less than 425 lb. of fat, only four (including two inbreds) would have been unborn through culling of a low-producing ancestor. Since the removal of a female because of low production means that ALL of her future female progeny are not part of the herd, it is disturbing to find that that part, which might be called unborn herd, has demonstrated the same average producing ability as that of the residual herd, made up of individuals with no cull female ancestors. Perhaps all of this could be summed up by saying that culling for low production has an effect on the average level of production in the immediate herd, but it does not improve the average ability of the future herd. This is no cause for discouraging dairymen from eliminating low-producing cows, but it does not warrant the belief that by so doing he is contributing to the improvement of his future herd. That improvement rests largely on the quality of the herd sires he uses.

As already stated, the problem of improving the production level is one which applies to the entire national dairy herd. As such, it is well to consider all approaches to that objective, and formulate a program which gives consideration to all rational procedures. In this enlightened age there is no reason to discuss crossbreeding as if it were a sinful practice. It is primarily a procedure for use of commercial dairymen who have no particular breed interest. It is in no way antagonistic to the interests of pedigreed cattle breeders. The modern recommendation is that cross-breeding be done with production-proved sires. It can be most helpful when used to change the average quality of milk in order to meet the market demand. This brings about a gradual change, but from a herd health standpoint it is a safer procedure than the purchase of animals. The hybrid vigor which results in first crosses can be of value in raising the herd level more quickly than by straight breeding. This gain can be sustained and added to by continuing to breed to production-proved sires.

It is well to bear in mind that regardless of what progress is made in improving our cattle genetically, this increased ability will only be expressed when the dairymen cooperate by providing a favorable environment and feed of good quality in sufficient quantity during the lactation.

This writer has no desire to oversimplify the complex problem of breeding better dairy cattle. Some of our teaching and extension work in this field might well be reviewed for the purpose of determining, in the light of our present knowledge, what recommendation can be made with a degree of confidence to dairymen and cattle breeders. It would appear that the part of the program which we offer to young people is too heavily weighted with training in judging and showing cattle. This is not career training, and tends to bring young dairymen into herd ownership with some faulty notions about the economics of the business they are engaged in. Might it not be well to start them off better equipped to judge the relative merits of earning capacity and good looks? They already know that one sells a cow only once, but sells her milk every day for 10 mo. of the year. Financial benefits from type are only realized when the animal or her progeny are sold. In a heifer-judging contest the winner is only the best looking animal at the particular moment—not necessarily the best earner. The tendency is to glamorize the winner unduly, and to disregard so many animals of almost equal pulchritude which stand below first place. It might be a good idea to reassemble some of these heifer classes after they are in production, then assess their true value. The whole idea here is to follow up what we are doing today to try to determine if it has any permanent value. Modern training may require a degree of competition to make it interesting, but it is possible that overemphasis may leave a lasting impression or result in later disillusionment. It is recognized that some progress is being made in introducing constructive modifications in judging contests.

We should be concerned mainly with bringing our young dairymen into their growing responsibilities with well-balanced knowledge of the many problems which confront them. Thus equipped, they are better able to cope with the daily trials, and will still have plenty of time to participate in the fall show season.

The time-worn procedure of pedigree selection offers only an even chance of success in improving the herd. Female selection affords no promise of adding much to the genetic worth of the residual herd. Therefore, it appears at this time that the proved-sire method of breeding will bring about herd improvement with greater certainty than any other procedure available to dairymen and breeders. Artificial breeding is ready to implement this breeding method to a high degree.

An adequate supply of dependably proved sires should be the concern of all who are interested in the future welfare of our national dairy herd. A rising level of production will increase the need for greater ability in bulls to be used in the future. All sources of well-endowed sires should be given encouragement

to continue their development. Good germ plasma like gold may be where it is found, but it would not be a bad idea to try to establish more places where it will be found. It is time that more consideration be given to the aims and purposes of dairy cattle breeding research. Plant breeders have always concerned themselves more with the production and development of living organisms than with a study of the methods whereby they are produced. The utilization of hybrid corn would have been very restricted if the results of research had been limited to an exposition of how various hybrids were developed. There is no reason why the product of well-planned and -executed dairy cattle breeding research should not be the breeding of stock which will help fill the need for capable bulls. This concept of breeding research should be helpful in winning support for a type of work which requires liberal financial aid. Unless our breeding research projects result in animals suitable for future herd improvement, they will fail in one of the main purposes of such research.

To sum up briefly, at this time it would appear that the use of high-level production-proved sires, artificially or otherwise, is the best recommendation which can be made for constructive breeding. Female selection should be practiced to improve the level of the existing herd. A comfortable environment and sufficient feed the year round (with emphasis on good-quality home-grown roughage) should be provided to enable cows to express a good inheritance.

ROSTER OF DAIRY TECHNOLOGY SOCIETIES[1]

H. F. JUDKINS, *Sec.-Treas. A.D.S.A.*
White Plains, New York

The Dairy Technology Societies in this country are an active group of influential people in the Dairy Industry. They come from all segments of the industry and represent many branches of specialization. They meet monthly over the nation and have speakers who discuss vital, timely, and practical problems that range from milk production on the farm through processing, automation, and ways to get better and more nutritious products to consumers.

The American Dairy Science Association is happy to publish this roster of Dairy Technology Societies, and joins hands with them in promoting the best interests of our great Dairy Industry.

ARIZONA
Arizona Dairy Technology Society
Dr. Warren Stull, Sec.
Dept. of Dairy Science
University of Arizona
Tucson, Arizona

COLORADO
Colorado Dairy Technology Society
Mr. Charles Dunlap, Sec.
955 11th Street
Denver, Colorado
Southern Colorado Dairy Tech. Soc.
Corky Bloise, Sec.
Sinton Dairy Co.
4001 N. Chestnut Street
Colorado Springs, Colorado

FLORIDA
North Florida Dairy Tech. Club
S. D. Williams
Pine Grove Dairy
2723 Cherrywood Road
Jacksonville, Florida

GEORGIA
Atlanta Dairy Technology Soc.
Prof. J. J. Sheuring, Sec.
Professor of Dairying
University of Georgia
Athens, Georgia

ILLINOIS
Central Illinois Dairy Society
Dr. S. L. Tuckey, Sec.
101 Dairy Manufactures Building
University of Illinois
Urbana, Illinois
Chicago Dairy Technology Soc.
Dr. Joseph Tobias, Sec.
103 Dairy Manufactures Building
University of Illinois
Urbana, Illinois
Southern Illinois Dairy Tech. Soc.
Prof. Howard H. Olson
Southern Illinois University
Carbondale, Illinois

INDIANA
Indianapolis Dairy Tech. Club
Dr. F. J. Babel
Dairy Department
Purdue University
Lafayette, Indiana
Michiana Dairy Technology Club
Mr. H. Scott Porter
1013 Louise Street
South Bend 15, Indiana
Southwestern Indiana Dairy Tech. Soc.
Mr. S. M. Gregory, Sec.
Room B-1, Court House
Evansville, Indiana

IOWA
Dubuque Dairy Tech. Society
Clem Walsh, Sec.
Dubuque, Iowa

KANSAS
Kansas Dairy Technology Soc.
Prof. T. J. Claydon, Sec.
Dept. of Dairy Husbandry
Kansas State College
Manhattan, Kansas

[1] This roster is as up-to-date as it was possible to make it at this time.

KENTUCKY
Tri-Cities Dairy Tech. Soc.
Mr. Cecil Derr
Fall City Dairy Cooperative
Louisville, Kentucky

MARYLAND
Maryland—D. C. Dairy Tech. Soc.
Mr. Robert Stout, Sec.
2210 N. Charles Street
Baltimore, Maryland

MICHIGAN
Central Michigan Dairy Tech. Soc.
Prof. J. M. Jensen, Sec.
Dairy Department
Michigan State University
East Lansing, Michigan
Detroit Dairy Technology Society
Prof. L. G. Harmon, Sec.
Michigan State College
East Lansing, Michigan
Western Michigan Dairy Tech. Soc.
Prof. D. L. Murray
Michigan State University
East Lansing, Michigan

MINNESOTA
Minnesota Dairy Technology Soc.
Dr. Howard Morris, Sec.
University of Minnesota
Dept. of Dairy Husbandry
Minneapolis, Minnesota

MISSOURI
Dairy Technology Society of Kansas City Area
Prof. W. H. E. Reid
University of Missouri
Columbia, Missouri
Dairy Technology Society of Greater St. Louis
J. Shields Christy
3 Buckeye Drive
Ferguson 21, Missouri

NEBRASKA
Nebraska Dairy Technology Society
Mr. Charles Fraley, Sec.
Roberts Dairy Co.
Lincoln, Nebraska

NEW JERSEY—NEW YORK
Metropolitan Dairy Technology Soc.
Dr. Samuel Lear, Sec.
Rutgers University
New Brunswick, New Jersey

NORTH CAROLINA
North Carolina Dairy Tech. Soc.
Prof. W. M. Roberts, Sec.
North Carolina State College
Raleigh, North Carolina

OHIO
Central Ohio Dairy Tech. Soc.
Prof. T. D. Harman, Sec.
Dept. of Dairy Technology
Ohio State University
Columbus, Ohio
Cincinnati Dairy Technology Soc.
Dr. T. D. Harman
Dept. of Dairy Technology
Ohio State University
Columbus, Ohio
Maumee Valley Dairy Tech. Soc.
Prof. Frank Koval, Sec.
Dept. of Dairy Technology
Ohio State University
Columbus, Ohio
Northeastern Ohio Dairy Tech. Soc.
Prof. Frank Koval, Sec.
Dept. of Dairy Technology
Ohio State University
Columbus, Ohio

OKLAHOMA
Oklahoma Dairy Technology Society
Prof. Paul E. Johnson, Sec.
Oklahoma State University
Stillwater, Oklahoma

PENNSYLVANIA
Tri-State Dairy Technology Soc.
Mr. P. F. Demblowski, Sec.
2017 Preble Avenue
Pittsburgh 3, Pennsylvania
Philadelphia Dairy Tech. Soc.
Mr. G. Donald Weber
Abbotts Dairies
16th and Tasker Streets
Philadelphia 46, Pennsylvania

TEXAS
Texas—New Mexico Dairy Tech. Soc.
Prof. E. R. Jarman, Sec.
Dept. of Dairy Industry
Texas Technological College
Lubbock, Texas

UTAH
Dairy Technology Association of Utah
Prof. Paul B. Larsen, Sec.
Utah State Agriculture College
Logan, Utah

WASHINGTON
Northeast Section
Harry J. Johnson
2734 West Longfellow
Spokane, Washington
Northwest Section
W. R. Knutzen
Box 243
Bellingham, Washington
Southeast Section
Jay H. Gano
3209 Webster Avenue
Yakima, Washington
Southwest Section
Jack Carlson
412 N. 14th Street
Elma, Washington

WEST VIRGINIA
West Virginia Dairy Tech. Soc.
Mr. Lawrence Menefee, Sec.
142 Sunset Drive
Charleston 1, West Virginia

WISCONSIN
Wisconsin Dairy Tech. Soc.
Prof. K. G. Weckel, Sec.
Dept. of Dairy Industry
University of Wisconsin
Madison, Wisconsin
Lacrosse Dairy Technology Soc.
Kenneth Schweigert, Sec.
Union Center, Wisconsin
The Eau Claire Dairy Tech. Soc.
Mr. Arthur C. Schlewitz
Sunlite Dairy
Eau Claire, Wisconsin
Illinois-Wisconsin Dairy Tech. Soc.
Mr. C. A. Davis, Sec.
Dept. of Health
Beloit, Wisconsin

OUR ASSOCIATION

AMERICAN DAIRY SCIENCE ASSOCIATION PAST-PRESIDENTS LOOK AHEAD

G. M. TROUT
Historian, American Dairy Science Association
Department of Dairy, Michigan State University, East Lansing

During the American Revolution Edmund Burke, in a letter to the English National Assembly, wrote, "You can never plan the future by the past." About the same time an American patriot, Patrick Henry, in a speech in the Virginia convention, March, 1775, emotionally exclaimed, "I know of no way of judging of the future but by the past." These compatriots expressed two opposite ideas.

Whether one can judge the future of the American Dairy Science Association by the actions of the past-presidents of the Association at their annual dinner, Union Building, North Carolina State College, Raleigh, June 15, 1958, remains to be seen. At any rate, the enthusiasm of the 17 of the 28 living past-presidents attending the dinner with the current Executive Board, present in full body, foretold a bright future for the Association.

Conspicuously absent in the discussion following the formal program were any allusions to the "good old days." Encouragement to the current officers seemed to be engendered by the very presence of such past-presidents as JOURNAL founder, Professor Emeritus Julius H. Frandsen, Massachusetts, two-year fourth president 1912–1913, and Charter Member, Professor Emeritus E. S. Guthrie, Cornell, 24th president, 1940.

Other past-presidents assembled for the occasion were H. C. Jackson, 1933; H. W. Gregory, 1938; H. F. Judkins, 1942; H. P. Davis, 1943; A. C. Dahlberg, 1944; A. C. Ragsdale, 1945; F. Ely, 1947; W. E. Peterson, 1949; G. M. Trout, 1950; R. B. Becker, 1951; H. B. Henderson, 1953; W. V. Price, 1954; L. A. Moore, 1955; I. A. Gould, 1956, and C. F. Huffman, 1957.

To the credit of the membership who elected them, the Executive Board was on duty and in full attendance. The list includes D. V. Josephson, president; K. L. Turk, vice-president; H. F. Judkins (past-president, 1942), secretary-treasurer; E. O. Herreid, JOURNAL editor-in-chief; C. F. Huffman (immediate past-president); and E. L. Fouts, I. W. Rupel, G. M. Werner, E. H. Parfitt, E. L. Jack, and H. A. Herman. The guest of honor was the newly elected vice-president, A. C. Fay, Florida, formerly Director of Laboratories and Research, H. P. Hood & Sons, Boston. The current officers and directors, comprising the Executive Board, which had been in almost continuous session on Association problems for the preceding 48 hours, seemed to welcome the opportunity to let "the pasts" have the limelight.

Following the North Carolina disjointed fried chicken and country ham plantation dinner, presiding Josephson laid the groundwork for a successful session through introductions and statement of plans for discussion. He then introduced past-president Walter Price, 1954, chairman of the one-year-old Policy Committee and asked him to make a full report of the Committee's deliberations. In addition to Price as chairman, this committee consisted of I. A. Gould, F. J. Arnold, J. H. Erb, R. E. Hodgson, and H. F. Judkins. Apparently, the formation of this Policy Committee resulted directly from suggestions made to that effect at one of the 1957 Executive Board meetings and summated at the previous past-presidents' dinner, Stillwater, June, 1957. Regardless of the source of spark which engendered its formation, the idea was a good one, as evidenced by the report. The Policy Committee has no power to advocate, promote, or publicize its findings; its opinions are reported to the Executive Board and that Board may accept or reject them in whole or in part. Among the areas of committee deliberation during 1957–58 were:

a) *Officer selection and development.* This is not easy. Time is involved in learning the obligations of office. The experience of the nominees must be carefully considered. Management of Association business affairs depends in large part upon the calibre of the directors.

b) *Acceptance of awards.* From time to time cash awards are proffered the Association for specific attainments. The Policy Committee considered their acceptance, addressing themselves as to the number, their administration, and the selection and field of possible recipients.

c) *Honorary recognition of Association members.* Price stated that such recognition for meritorious, long-time service to the Association gave "a marvelous lift to those receiving it." Accordingly, the Annual Association Award was considered. A slight change in wording of this award by the committee seemed to make it more significant. The Policy Committee recommended that the Association Award hereafter be designated as the American Dairy Science Association Award of Honor.

d) *Life membership.* The Committee recommended that life membership be bestowed upon those having been members of the Association for 40 years, as well as upon those who, upon their retirement, have been members for 25 years or more.

e) *Recognition of outstanding service of younger members.* The Committee recognized the need for encouragement of the younger members who, through service, were showing more-than-usual interest in the Association. Perhaps a suitable scroll, plaque, or citation might not be out of order. No definite recommendations were made.

f) *Reorganization and/or renaming of Association sections.* Some thought was given to renaming the extension, manufacturing, and production sections as divisions. Research, teaching, and extension were more or less common to all. This problem is still under consideration.

g) *Possibility of holding an International Dairy Science Congress, 1962.* This would be held in Washington, D. C., and at the University of Maryland, in conjunction with the celebration of the 100th anniversary of the passage of the Land Grant Act, 1862, and the foundation of the United States Department of Agriculture. The Board appointed a special committee to develop this idea as far as possible.

h) *Problems of integration through correspondence.* The Policy Committee carried on its activities through correspondence. While attempting to crystallize its thoughts on certain matters, the Committee came to the realization of the difficulties encountered and the patience required of all members to arrive at just conclusions. The problem of reading letters, especially between the lines, to get the writers' feelings on the matter, was no easy task.

President Josephson opened the meeting for discussion. General lack of sustained discussion on further Association problems by the past-presidents seemed to give a sanction and blessing to the objectives of the Policy Committee beyond the expression of appreciation for its work for the Association.

President Josephson reappointed the present membership to the Policy Committee for another year, with Price again as chairman. Josephson charged the committee "to continue to think about the present and future problems of the Association."

Other business consisted in a brief report of Association affairs by President Josephson. The past-presidents were pleased to learn of the *a*) excellent financial condition of the Association; *b*) all-time-high membership roster and subscription list; *c*) and the results of the balloting regarding the future of dairy abstracts. The details will be given in the report of the Executive Board.

Judging from this meeting, one may say in conclusion that the past-presidents' annual gathering is neither an occasion for looking backward nor of bewailing the shortcomings of the current officers, but rather an occasion for radiating a sustaining influence to those to whom the business affairs of the American Dairy Science Association are entrusted.

ANNUAL REPORT

COMMITTEE ON JUDGING DAIRY PRODUCTS, A.D.S.A.
Raleigh, North Carolina, June, 1958

The Committee on Judging Dairy Products, A.D.S.A., under the direct supervision of C. J. Babcock, assisted by D. R. Strobel, U. S. Department of Agriculture, supervised the 36th Collegiate Students' International Contest in the Judging of Dairy Products, San Francisco, California, October 21, 1957, in connection with the Milk Industry Foundation and International Association of Ice Cream Manufacturers' Conventions. This was the 23rd contest sponsored jointly by the American Dairy Science Association and the Dairy Industries Supply Association, and the 36th for the American Dairy Science Association. Twenty-one college teams participated in 1957. They were from California (Polytechnic), California (University), Colorado, Connecticut, Fresno State, Idaho, Iowa, Kansas, Minnesota, Mississippi, Missouri, Nebraska, Ohio, Oklahoma, Oregon, Pennsylvania, Texas Tech, Utah, Washington, Wisconsin, and Wyoming. For the first time, Fresno State College and the University of Wyoming entered teams in the Collegiate Students' International Contest in the Judging of Dairy Products. This brings to 46 the number of colleges participating from the beginning of the contest, 42 yr. ago.

The official judges for butter, cheese, milk,

DAIRY INDUSTRIAL FELLOWS, 1957

Two fellowships were offered in 1957, carrying stipends of $1,800 and $1,650. These fellowships were assigned as follows:

Team	Rank in contest	Name of senior * recommended for fellowship	School selected for graduate work
Univ. of Connecticut Storrs, Connecticut Dr. W. A. Cowan, Head Dept. Animal Industries	First	Edward P. Geishecker 301 Savin Hill Avenue Dorchester, Massachusetts	Ohio State University Columbus 10, Ohio Dr. I. A. Gould, Chairman Dept. of Dairy Technology
State College of Washington Pullman, Washington Dr. A. O. Shaw, Chm. Dept. of Dairy Science	Second	Donald H. Raistakka 604 California Street Pullman, Washington	Oregon State College Corvallis, Oregon Dr. G. A. Richardson Dept. Food and Dairy Tech.

* Any senior (not necessarily a team member) majoring in dairy industry from the winning school is eligible for consideration for the award.

and ice cream were N. E. Fabricius, H. L. Wilson, D. A. Pettee, and J. H. Erb, respectively.

The ten high-ranking teams in judging of products ranked as follows:

1. University of Connecticut
2. State College of Washington
3. Kansas State College
4. Ohio State University
5. University of Missouri
6. University of Minnesota
7. University of California

T8. University of Wisconsin

T9. Iowa State College

10. University of Nebraska

The complete report of the ten high-ranking teams and individuals in the judging of butter, cheese, milk, ice cream, and all products was published in the JOURNAL OF DAIRY SCIENCE, December, 1957.

MISCELLANEOUS

Joint meetings with D.I.S.A. During the year, the committee held a joint meeting with the Contest and Fellowship Committee of the Dairy Industries Supply Association at Stillwater, Oklahoma. Plans were discussed for furthering the best interests of the contest and the Dairy Industrial Fellowship program.

Solicitation of coach-comments. Following the 1957 contest, coach-comments concerning the contest again were solicited. Coaches' comments were coded, assembled, and studied, with the aim of improving the 1958 contest in any way possible. The Committee was gratified at their frank responses, especially at the assurances that the contest with all its affiliations was fulfilling its functions satisfactorily.

1958 contest rules. Upon recommendation of the Committee, some drastic changes were made in the 1958 rules. These changes consisted primarily in placing greater responsibility upon the official judges of the various products and the superintendent of the contest. Through the D.I.S.A. office, the 1958 rules were mimeographed and sent to the coaches and heads of the Dairy Departments of the United States and Canada.

Status of research under fellowship grants. Through the efforts of Mrs. Beatrice Prescott, Secretary, Contest and Fellowship Committee, D.I.S.A., and the full cooperation of the Dairy Departments involved, the research program under the fellowship grants is kept very active and its publications are kept up-to-date. Twenty-nine fellowships have been processed from 1947 (resumption of contests following World War II) to 1957, inclusive, bringing the total number of fellowships to 85 since the program was inaugurated in 1930.

Committee changes. During the year, the Committee lost a loyal, long-time, valued member in the passing of C. J. Babcock, who served faithfully and so well the interests of dairy products judging since 1925. Such experience as "C. J." brought to the Committee can not be replaced. Nevertheless, it is hoped that within a short time a person will be appointed to fill the vacancy resulting from his passing.

Record of winning teams and individuals. During the past year the Committee completed the roster of winning team members and individuals in the judging of each product since the inception of the contest in 1916. This was an arduous task, made so, in part, because of inadequate records within some departments. The complete records are appended to this report.

Roster of all team members since 1930. An attempt was made during the past year to complete the roster of all team members since 1930, which was inaugurated some years ago. This involved getting the names, occupations, and addresses of 1,464 men and women, the number of contestants from 1930 to 1957, in-

clusive. College departments lent full cooperation. Obviously, due to loss of contact, 100% completion of such a roster was not possible. Nevertheless, the records are sufficiently complete that a study was made on type of employment within and without the dairy industry. This information should be made available within the year.

Questionnaire to former contestants. A questionnaire and ballot envelope were sent to team members from 1930 to 1956. A very high yield of returns furnish excellent, pertinent information, invaluable to college training programs. The Committee hopes to analyze these returned questionnaires and prepare a paper for publication resulting from this study.

History of contest. The contest history has been brought up to date and is all-inclusive from 1916 to 1957. The complete history is now in the D.I.S.A. office, where it is being prepared for publication. Through the generosity of D.I.S.A. it will be made available for the Honors Banquet, Chicago, December 1958.

Other studies. Studies were inaugurated looking into the feasibility of using IBM cards for contestant judging. This study is in progress and will be reported later.

A subcommittee to develop a score card for dry milk, E. L. Thomas, Chairman, has made a real contribution in their studies. This subcommittee will make a separate report.

Cooperation with industry. The Committee has worked closely with members of the Contest and Fellowship Committee, D.I.S.A., and appreciates fully this fine relationship. The D.I.S.A. committee members are:

Charles Weinreich, Cherry-Burrell Corporation, *Chairman*
George Huffman, Ex-Cell-O Corporation, Pure-Pak Division, *Vice-Chairman*
C. A. M. Anderson, The King Company
Neil C. Angevine, Meyer-Blanke Company
Virginia Jones Baker, *Western Dairy Foods Review*
T. A. Burress, The Heil Company
Charles O. Davis, Jr., Walker Stainless Equipment Company
Clark Derleth, Kusel Dairy Equipment Company
W. S. Feagan, Midwest Creamery and Dairy Supply Company
Gordon A. Houran, The DeLaval Separator Company
Sherman Little, Sr., Weber Showcase & Fixture, Inc.
Robert L. Lloyd, American Maize-Products Company
John A. Miller, Chester-Jensen Company
Norman Myrick, *American Milk Review*
Barney B. Parker, Pangburn Company, Inc.
Dan Roahen, The Creamery Package Mfg. Company
V. K. Shuttleworth, American Can Company
H. P. Smith, Wm. J. Stange Company
V. K. Wilt, Liquid Carbonic Corporation, Standard Vanilla Division
A. C. Woodruff, Mojonnier Bros. Company

Conclusion. The Committee on Judging Dairy Products, A.D.S.A., is grateful for the full cooperation of the many called upon for specific information and suggestions during the past year. The Committee is deeply appreciative of the support and help of the Dairy Industries Supply Association and its Contest and Fellowship Committee. The Committee wishes to thank especially Mrs. Beatrice Prescott, Secretary, Contest and Fellowship Committee, D.I.S.A., for her invaluable service and cooperation, not only to the Committee, but for the fine contribution of good will she is engendering in the minds of the collegiate students participating in the contest, who will become the dairy leaders of tomorrow.

Respectfully submitted,

C. J. Babcock (deceased)
Leonard R. Dowd
P. A. Downs
E. L. Thomas
F. G. Warren
J. J. Willingham
G. M. Trout, Michigan State University, East Lansing, *Chairman, Committee on Judging Dairy Products, American Dairy Science Association*

The winning team and high individual in judging CHEESE in the Collegiate Students' International Contest in the Judging of Dairy Products, by years, 1916-1957 inclusive

Year	Winning team			High individual	
	School	Coach	Members	School	Name
1916	(Cheese was not included until 1917)				
1917	Nebraska	J. H. Frandsen	Eli Duncombe J. D. McKelvey J. B. Shepherd	Nebraska	Eli Duncombe
1918	*(No contest, due to World War I)*				
1919	Ohio	D. S. Kochheiser	G. M. Boehm R. Kennedy J. Pontius	Maryland	Thomas Downin
1920	Ohio	D. S. Kochheiser	T. V. Armstrong H. J. Kline A. H. Neu	Maryland	Clayton Reynolds

1921	Ohio	R. B. Stoltz	C. C. Darnell H. R. Jones H. J. Krohm	Ohio	H. R. Jones
1922	Ohio	R. B. Stoltz	E. M. George T. W. Lawler W. O. Christopher	Ohio	W. O. Christopher
1923	Ohio	R. B. Stoltz	G. Lewis Howard McClarren J. C. Skidmore	Pennsylvania	William F. Borst
1924	Purdue	H. W. Gregory	G. T. Badger P. H. Cherry C. A. Keene	Purdue	P. H. Cherry
1925	Iowa	M. Mortensen	Snowden R. Clary Walter L. Olson Ralph P. Wilson	Iowa	Snowden R. Clary
1926	S. Dakota	C. C. Totman	E. O. Herreid John Nelson John Steele	S. Dakota	John Steele
1927	Tennessee	T. B. Harrison	L. O. Colebank S. A. Hinton E. S. Permenter	Iowa	Russell R. Wood
1928	W. Virginia	G. M. Trout	W. R. Barnard W. Harold Wayman S. J. Weese	W. Virginia	W. R. Barnard
1929	Ohio	R. B. Stoltz	Willard C. Boyer Gale R. Kasler Luthur Mindling	Ohio	Gale R. Kasler
1930	Kansas	W. H. Martin	Walter W. Babbitt Ralph A. Germann John L. Wilson	Kansas	Ralph A. Germann
1931	Michigan	G. M. Trout	Carl E. Haradine Donald Keppel Maurice Poncin	Michigan	Maurice Poncin
1932	Michigan	G. M. Trout	Merle Dean Gordon Dickerson Harold Harrison	Nebraska	Perry Meridith
1933	Wisconsin	L. C. Thomsen	Glenn H. Hagberg Walter Uphoff Fred Wagner	Nebraska	William Ralston
1934	Iowa	N. E. Fabricius	W. H. Brown H. M. Jensen R. F. Sailer	Arkansas	William E. Shaw
1935	Mississippi	F. H. Herzer	J. Bryant R. O. Marion H. W. White	Ohio	H. P. Smith
1936	Tennessee	T. B. Harrison	William M. Roberts H. N. Carringer R. K. Kilpatrick	Tennessee	William M. Roberts
1937	Minnesota	S. T. Coulter	Brooks Naylor Goodwin Sonstegaard Leonard Sullivan	Wisconsin	Rudolph P. Zelm
1938	Cornell	E. S. Guthrie	Nathan Andersky Carlton O. Blackmore Varnum D. Ludington	Mississippi	L. M. McCalla
1939	Wisconsin	L. C. Thomsen	Richard Ames Herbert A. Hollender Owen Pilgrim	Tennessee	Marshal C. Winton
1940	Mississippi	F. H. Herzer	Luke Davis J. L. Pointowski A. B. Womble	Mississippi	Luke Davis
1941	Michigan	I. A. Gould	Kenneth M. Dunn Percy J. Smeltzer Robert E. Stout	Michigan	Robert E. Stout
1942–1946	*(No contests, due to World War II)*				
1947	Mississippi	F. H. Herzer	Charles M. Betterton Lott B. Smith Leslie O. Templeton	Mississippi	Charles M. Betterton
1948	Michigan	J. M. Jensen	Lowell A. Allen Donald R. Moore Ralph Whitehead	Pennsylvania	William J. Deisley

1949	Mississippi	F. H. Herzer	Dee M. Graham Richard J. Stanley Sam L. Swett	Mississippi	Sam L. Swett
1950	Iowa	W. S. Rosenberger	Duane Osam William E. Sandine Edward Schuch	Mississippi	Thomas E. Gilmore
1951	Iowa	W. S. Rosenberger	Delmar A. Andersen Calvin Bolton A. A. Christensen	Iowa	A. A. Christensen
1952	Ohio	W. L. Slatter	Kenneth Huffman Bert W. Taylor Edward J. Haynes, Jr.	Ohio	Bert W. Taylor
1953	Cornell	W. F. Shipe	Norman H. Dobert B. A. Schlag W. F. Witter	Cornell	Norman H. Dobert
1954	Tennessee	H. N. Carringer	R. W. Johnson Walter W. Johnson Richard D. Martin	Kansas	Wm. H. Bergman
1955	Iowa	W. S. Rosenberger	Herbert Hakes H. John Pollei James Riekens	Georgia	Walter E. Verity
1956	Iowa	W. S. Rosenberger	Robert C. Hakeman Charles M. Mead Thomas Ruzicka	Kansas	Thomas E. Quint
1957	Kansas	W. H. Martin	Allen Millen Harold Suelter T. W. Townsend	California (Fresno State)	Robert L. West

The winning team and high individual in judging BUTTER in the Collegiate Students' International Contest in the Judging of Dairy Products, by years, 1916-1957 inclusive

	Winning team			High individual	
Year	School	Coach	Members	School	Name
1916	Pennsylvania	E. L. Anthony	B. R. Ober Silas Page E. R. Taylor	Pennsylvania	Silas Page
1917	S. Dakota	C. Larson	S. V. Layson A. E. Miller A. Tompkins	S. Dakota	A. E. Miller
1918	*(No contest, due to World War I)*				
1919	Iowa	M. Mortensen	R. O. Linder H. R. Schultz Paul Watson	Maryland	Tomas Downin
1920	S. Dakota	T. H. Wright	George Biggar Richard Gardener Dave Kopland	Maryland	J. H. Snyder
1921	Ohio	R. B. Stoltz	C. C. Darnell H. R. Jones H. J. Krohm	Iowa	Peter Toens
1922	S. Dakota	T. M. Olson	Glenn G. Prunty Carrol Wallis Floyd Wright	Oregon	D. McKenzie
1923	Pennsylvania	W. M. Martin	William F. Borst Verne K. Heckel Mento P. Saponis	Pennsylvania	Verne K. Heckel
1924	Iowa	M. Mortensen	Warner W. Griese Fern T. Moore Roy O. Storvick	Iowa	Roy O. Storvick
1925	S. Dakota	C. C. Totman	Emery Bartle Daniel Jacobsen Sidney Quam	Iowa	Walter L. Olson
1926	Oregon	V. C. Chappel	W. B. Cooper A. Gribskov D. R. Winn	Oregon	D. R. Winn
1927	Iowa	B. W. Hammer	Delbert Breazeale N. E. Fabricius Russell R. Wood	Oregon	Alvin W. Hare
1928	Iowa	E. F. Goss	Ralph S. Farmer Gilbert Myers K. Snyder	Mississippi	H. S. Burford

1929	Oregon	G. H. Wilster	W. H. Brands Byron Gordon Melville Richey	Oregon	Byron Gordon
1930	Mississippi	F. H. Herzer	J. D. Quinn R. L. Newman Boyce Walker	Mississippi	Boyce Walker
1931	Mississippi	F. H. Herzer	T. B. Stokes J. H. Trotter B. H. Whitfield	Iowa	O. H. Ause
1932	Purdue	H. W. Gregory	R. W. Cantley R. F. Logan K. E. Mennen	Purdue	R. F. Logan
1933	S. Dakota	C. C. Totman	Forest Gary George Sanderson Delmar Spicer	S. Dakota	Delmar Spicer
1934	Minnesota	S. T. Coulter	C. Raymond Hoglund Donald V. Josephson Harold E. Meister	S. Dakota	Sig. Svanoe
1935	Tennessee	T. B. Harrison	C. H. Detcheon J. C. Flake H. M. Hayes	Tennessee	H. M. Hayes
1936	Iowa	N. E. Fabricius	E. M. Barker John T. Griffeth W. H. Hoecker	Iowa	John T. Griffeth
1937	Nebraska	P. A. Downs	Oakley Larson Don O. Radenbaugh Arell J. Wasson	Kansas	Wayne Klamm
1938	Iowa	N. E. Fabricius	Joe Levine Leland G. Sherman Thomas S. Warner	Iowa	Thomas S. Warner
1939	Iowa	N. E. Fabricius	Raymond J. Nelson Oerel M. Russell Donald E. Sherman	Iowa	Raymond J. Nelson
1940	Minnesota	S. T. Coulter	Raymond Mykleby Oleen Sonstegaard Lennie Underdahl	Minnesota	Oleen Sonstegaard
1941	Connecticut	E. O. Anderson L. R. Dowd	Leonard Goldberg Richard A. Marland Herman L. Neiditz	Connecticut	Leonard Goldberg
1942–1946	*(No contests, due to World War II)*				
1947	Connecticut	E. O. Anderson L. R. Dowd	Evelyn H. Fish Donald D. Martin Alan F. Tobie	Connecticut	Alan F. Tobie
1948	Iowa	W. S. Rosenberger	Charles H. Fitch Arnold D. Nelson Donald H. Pflueger	Iowa	Donald F. Pflueger
1949	Minnesota	Elmer Thomas	Russell J. Moe Raymond G. Otto George Vale	Minnesota	Russell J. Moe
1950	Iowa	W. S. Rosenberger	Duane Osam William Sandine Edward Schuch	Tennessee	Farris E. Ashe
1951	Iowa	W. S. Rosenberger	Delmar A. Andersen Calvin Bolton A. A. Christensen	Michigan	William F. Kruger
1952	Cornell	W. F. Shipe, Jr. J. E. White	Henry N. Charlap John A. Tarr Cliford J. Wengert	Iowa	Carl Zurborg
1953	Ohio	W. L. Slatter	Jack H. Fowler Ralph L. Paul Robert C. Milkie	Ohio	Robert C. Milkie
1954	Iowa	W. S. Rosenberger	R. S. Goodale R. J. Hendrick W. H. Stoll	Michigan	Eugene Scott
1955	Tennessee	H. M. Carringer	J. V. Britton John R. Guice Robert Purvis	Mississippi	L. B. Barton
1956	Ohio	W. L. Slatter	Richard F. Flowers Gene L. Krajnak Robert Oberlin	Minnesota	Fred Dryg
1957	Connecticut	L. R. Dowd	Ernest Fleuette Edward Geisheeker Peter Schoeck	Iowa	David Duda

The winning team and high individual in judging MILK in the Collegiate Students' International Contest in the Judging of Dairy Products, by years, 1916-1957 inclusive

	Winning team			High individual	
Year	School	Coach	Members	School	Name
1916	(Butter only judged in 1916)				
1917	S. Dakota	C. Larsen	S. V. Layson A. E. Miller A. Tompkins	Ohio	D. S. Kochheiser
1918	*(No contest, due to World War I)*				
1919	S. Dakota	T. H. Wright	Rudolph Bucholz Dave Gilkerson Bernard Iverson	S. Dakota	Bernard Iverson
1920	Ohio	D. S. Kochheiser	T. V. Armstrong H. J. Kline A. H. Neu	Iowa	Clifford Farr
1921	Ohio	R. B. Stoltz	C. C. Darnell H. R. Jones H. J. Krohm	Arkansas	M. D. Johnson
1922	Massachusetts	H. F. Judkins	G. H. Brewer Joseph Goldstein Allan J. Heath	Massachusetts	G. H. Brewer
1923	Ohio	R. B. Stoltz	G. Lewis Howard McLarren J. C. Skidmore	Ohio	J. C. Skidmore
1924	Massachusetts	H. F. Judkins	Leland L. Currier Preston J. Davenport Irwin S. Sheridan	Iowa	W. W. Griese
1925	W. Virginia	G. M. Trout	G. A. Bowling L. L. Lough M. P. Ott	Ohio	W. B. S. Woodward
1926	Iowa	M. Mortensen	J. A. Earle G. L. Myers J. W. Tatum	Ohio	W. P. Stoops
1927	Kansas	W. H. Martin	E. Wayne Frey Elmer F. Hubbard Clarence O. Jacobson	Massachusetts	Leo Allen
1928	S. Dakota	C. C. Totman	Erwin Bartle Louis Eberlein H. C. Olson	Iowa	Gilbert Myers
1929	Ohio	R. B. Stoltz	Willard C. Boyer Gale R. Kasler Luther Mindling	Ohio	Gale R. Kasler
1930	W. Virginia	L. M. Thurston	W. F. Coull I. A. Gould Harry Trelogan	W. Virginia	Harry Trelogan
1931	Iowa	B. W. Hammer	D. L. Allen O. H. Ause C. L. Wilson	Vermont	Charles L. Park, Jr.
1932	Purdue	H. W. Gregory	R. W. Cantley R. F. Logan K. E. Mennen	Purdue	R. W. Cantley
1933	Illinois	P. H. Tracy	Willard J. Corbett Vergil N. Simmons R. T. Kenney	Illinois	Willard J. Corbett
1934	Mississippi	F. H. Herzer	R. C. Cunningham W. C. Patton H. G. Smith	Arkansas	William E. Shaw
1935	Tennessee	T. B. Harrison	C. H. Detcheon J. C. Flake H. M. Hayes	Ohio	Ross J. Winning
1936	Ohio	L. H. Burgwald	Joseph Adams Russell Fifer James B. Hamilton	Ohio	Russell Fifer
1937	S. Dakota	C. C. Totman	David Henry Henry A. Lardy Beeman Mullinix	S. Dakota	David Henry
1938	Ohio	L. H. Burgwald	Arthur D. Kasler Lowell Shook Wayne E. Smith	Illinois	Howard Kizer

1939	Iowa	N. E. Fabricius	Raymond J. Nelson Ocrel M. Russell Donald Sherman	Iowa	Donald Sherman
1940	Iowa	N. E. Fabricius	Dorrance I. Anderson John R. Jackson Claude J. March	Iowa	D. I. Anderson
1941	Ohio	L. H. Burgwald	Fred W. Carver James R. Ebright John R. Kohl	Ohio	James R. Ebright
1942–1946	*(No contests, due to World War II)*				
1947	Connecticut	E. O. Anderson L. R. Dowd	Evelyn H. Fish Donald D. Martin Alan F. Tobie	Michigan	Richard Huckins
1948	Iowa	W. S. Rosenberger	Charles H. Fitch Arnold D. Nelson Donald H. Pflueger	Pennsylvania	William J. Deisley
1949	Kansas	W. H. Chilson	Owen Fennema Harold A. Ramsey James H. Sherrod	Kansas	James H. Sherrod
1950	Connecticut	E. O. Anderson L. R. Dowd	Graham E. Hall Willis E. Parkin Stanley L. Ruxton	Connecticut	Willis E. Parkin
1951	Mississippi	F. H. Herzer	Robert A. Brooks Lawrence D. Custer Claude J. Blackburn	Michigan	Albert W. Hosner
1952	N. Carolina	Fred G. Warren	Ronald A. Chambers J. W. Sycock D. Ronald Biggerstaff	Pennsylvania	Charles Shear
1953	Ohio	W. L. Slatter	Jack H. Fowler Robert C. Milkie Ralph L. Paul	Ohio	Ralph L. Paul
1954	N. Carolina	Fred G. Warren	George W. Ellis Alva E. Williams Carol M. Yarborough	N. Carolina	Alva E. Williams
1955	Connecticut	L. R. Glazier	Warren S. Clark Herbert S. Hall Edwin H. McKinlay	Pennsylvania	Richard L. Mong
1956	Wisconsin	Myron P. Dean	William Breene Lyle Suprise Gerald Weiner	Wisconsin	Lyle Suprise
1957	Nebraska	P. A. Downs	Martin Carrancedo Maynard Harr Rodney Knox	Kansas	T. W. Townsend

The winning team and high individual in judging ICE CREAM in the Collegiate Students' International Contest in the Judging of Dairy Products, by years, 1916-1957 inclusive

	Winning team			High individual	
Year	School	Coach	Members	School	Name
1916–1925	(Ice cream not included in the contest)				
1926	Iowa	C. A. Iverson	J. A. Earle G. L. Myers J. W. Tatum	S. Dakota	John Nelson
1927	Massachusetts	M. J. Mack	Leo Allen R. Gordon Murch Walter B. Van Hall	Massachusetts	Leo Allen
1928	Ohio	R. B. Stoltz	Henry Apple Robert P. Isaly Irvin Krill	Massachusetts	Harold S. Adams
1929	Iowa	C. A. Iverson	Max C. Escher W. M. Tweeten W. C. Edwards	Purdue	Raymond C. Young
1930	Illinois	P. H. Tracy	H. A. Ackerman O. E. Ross D. E. Trummel	Ontario	Donald B. Goodwillie
1931	Vermont	J. A. Newlander	H. J. Leach Charles L. Park, Jr. C. E. Wright	Michigan	Carl E. Haradine
1932	Mississippi	F. H. Herzer	J. H. Long J. R. Mapp D. M. Smith	Cornell	Daniel A. Paddock

1933	Massachusetts	H. G. Lindquist	R. T. Coleman J. P. Edney Harry Pyenson	Michigan	Clare E. Monroe
1934	Ohio	J. H. Erb	D. Creamer W. L. Slatter Orvin Traver	Illinois	Perry E. Piper
1935	Nebraska	P. A. Downs	Harold Larson James Warner Stanley Whitson	Cornell	Charles Jennings
1936	Cornell	E. S. Guthrie	Charles A. Storie Albert S. Tomlinson John B. Wilcox	Cornell	Albert S. Tomlinson
1937	Ohio	J. H. Erb	J. Atlee Miller George E. Neeley Dan C. Roahen	Ohio	Dan C. Roahen
1938	Cornell	E. S. Guthrie	Nathan Andersky Carlton O. Blackmore Varnum D. Ludington	Mississippi	L. M. McCalla
1939	Iowa	N. E. Fabricius	Raymond J. Nelson Oerel M. Russell Donald E. Sherman	Iowa	Oerel M. Russell
1940	Connecticut	E. O. Anderson L. R. Dowd	John W. Atwood Henry Bentley John A. Bierkan	Minnesota	Oleen Sonstegaard
1941	Connecticut	E. O. Anderson L. R. Dowd	Leonard Goldberg Richard Marland Herman L. Neiditz	Connecticut	Leonard Goldberg
1942–1946	*(No contests, due to World War II)*				
1947	Iowa	W. S. Rosenberger	Robert F. Anderson Ted Brunner Herluf M. Peterson	Iowa	Ted Brunner
1948	Tennessee	T. B. Harrison	Delmar A. Boyce George H. Brink Harold McCracken	Iowa	Donald H. Pflueger
1949	Iowa	W. S. Rosenberger	Herbrt Ruggles George L. Weir Robert K. Wight	Connecticut	Roger W. Hunt
1950	Mississippi	F. H. Herzer	Richard H. Andrews Jack Davis Thomas E. Gilmore	Mississippi	Richard H. Andrews
1951	Cornell	F. Kosikowski F. Shipe	W. G. Hoffmann Roderigo Montealegre George M. Kloser	Texas A & M	S. R. Allen
1952	Massachusetts	D. H. Nelson	Gwendolyn Judson Marilea Papalia Leonard M. Libbey	Minnesota	Robert R. Farrar
1953	Ohio	W. L. Slatter	Jack H. Fowler Robert C. Milkie Ralph L. Paul	Kansas	Carl Richard Myers
1954	Kansas	W. H. Martin	Wm. H. Bergman Robert E. Lundquist Donald C. Vell	Texas Tech.	Charles Wales
1955	S. Dakota	R. J. Baker	Charles W. Sapp Kenneth Seas Kenwood Woldt	Ohio	Ronald Perkins
1956	Maryland	Alan Day	Lloyd C. Ludy Ronald Sappington Wilton Zaiser	Maryland	Ronald Sappington
1957	Ohio	W. L. Slatter	Gene Christman Charles Neikirk Larry Rayner	Washington	Raymond Bay

The winning team and high individual in judging ALL PRODUCTS in the Collegiate Students' International Contest in the Judging of Dairy Products, by years, 1916–1917 inclusive

	Winning team			High individual	
Year	School	Coach	Members	School	Name
1916	(Butter was the only product used in the first contest. ALL PRODUCTS award was not given)				
1917	(No rating for team ALL PRODUCTS, although butter, cheese, and milk were judged)			S. Dakota	A. E. Miller

1918	*(No contest, due to World War I)*				
1919	S. Dakota	T. H. Wright	Rudolph Bucholz Dave Gilkerson Bernard Iverson	S. Dakota	Rudolph Bucholz
1920	Ohio	D. S. Kochheiser	T. V. Armstrong H. J. Kline A. H. Neu	Ohio	A. H. Neu
1921	Ohio	R. B. Stoltz	C. C. Darnell H. R. Jones H. J. Krohm	Ohio	H. R. Jones
1922	Ohio	R. B. Stoltz	E. M. George T. W. Lawler W. O. Christopher	Ohio	E. M. George
1923	Pennsylvania	W. H. Martin	William F. Borst Verne K. Heckel Mento P. Saponis	Pennsylvania	William F. Borst
1924	Iowa	M. Mortensen	Warner W. Griese Fern T. Moore Roy O. Storvick	Iowa	Warner W. Griese
1925	Iowa	M. Mortensen	Snowden R. Clary Walter L. Olson Ralph P. Wilson	Pennsylvania	W. W. Richman
1926	Iowa	C. A. Iverson	J. A. Earle G. L. Myers J. W. Tatum	Iowa	J. W. Tatum
1927	Iowa	B. W. Hammer	Delbert Breazeale N. E. Fabricius Russell R. Wood	Massachusetts	Leo Allen
1928	Iowa	E. F. Goss	Ralph S. Farmer Gilbert Myers K. Snyder	Nebraska	Gene Spangler
1929	Ohio	R. B. Stoltz	Willard C. Boyer Gale R. Kasler Luther Mindling	Ohio	Gale R. Kasler
1930	Kansas	W. H. Martin	Walter W. Babbitt Ralph A. Germann John L. Wilson	Illinois	O. E. Ross
1931	Iowa	N. E. Fabricius	D. L. Allen O. H. Ause C. L. Wilson	Iowa	O. H. Ause
1932	Mississippi	F. H. Herzer	J. H. Long J. R. Mapp D. M. Smith	Mississippi	D. M. Smith
1933	Ohio	L. H. Burgwald	Richard Buchler George Mapp David Meyer	Illinois	Willard J. Corbett
1934	Ohio	J. H. Erb	Dwight Creamer Walter L. Slatter Orvin Traver	Arkansas	William E. Shaw
1935	Mississippi	F. H. Herzer	J. Bryant R. O. Marion H. W. White	Cornell	Charles Jennings
1936	Ohio	L. H. Burgwald	Joseph Adams Russell Fifer James B. Hamilton	Ohio	Joseph Adams
1937	Ohio	J. H. Erb	J. Atlee Miller George E. Neeley Dan C. Roahen	Ohio	Dan C. Roahen
1938	Cornell	E. S. Guthrie	Nathan Andersky Carlton O. Blackmore Varnum D. Ludington	Mississippi	L. M. McCalla
1939	Iowa	N. E. Fabricius	Raymond J. Nelson Oerel M. Russell Donald E. Sherman	Iowa	Donald E. Sherman
1940	Iowa	N. E. Fabricius	John R. Jackson Claude J. March Dorrance I. Anderson	Minnesota	Oleen Sonstegaard
1941	Ohio	L. H. Burgwald	Fred W. Carver James R. Ebright John R. Kohl	Ohio	John R. Kohl
1942–1946	*(No contests, due to World War II)*				

1947	Connecticut	E. O. Anderson L. R. Dowd	Evelyn H. Fish Donald D. Martin Allan F. Tobie	Iowa	Ted Brunner
1948	Iowa	W. S. Rosenberger	Charles H. Fitch Arnold N. Nelson Donald H. Pflueger	Michigan	Donald R. Moore
1949	Mississippi	F. H. Herzer	Dee M. Graham Richard J. Stanley Sam L. Swett	Iowa	Herbert Ruggles
1950	Iowa	W. S. Rosenberger	Duane Osam William E. Sandine Edward Schuch	Iowa	Edward Schuch
1951	Mississippi	F. H. Herzer	Robert A. Brooks Lawrence D. Custer Claude J. Blackburn	Iowa	A. A. Christensen
1952	Ohio	W. L. Slatter	Kenneth Huffman Bert W. Taylor Edward J. Haynes, Jr.	Ohio	E. J. Haynes, Jr.
1953	Ohio	W. L. Slatter	Jack H. Fowler Robert C. Milkie Ralph L. Paul	Ohio	Jack H. Fowler
1954	Kansas	W. H. Martin	Donald C. Vell William H. Bergman Robert E. Lundquist	Minnesota	John R. Dayle
1955	Mississippi	E. W. Custer	L. B. Barton Joe A. Sims Bobby G. Witt	Mississippi	Joe A. Sims
1956	Illinois	Joseph Tobias	Gordon Schnepper Fred J. Steffens F. C. Jaronitzky, Jr.	Illinois	F. C. Jaronitzky, Jr.
1957	Connecticut	L. R. Dowd	Ernest Fleuette Edward Geisheeker Peter Schoeck	Connecticut	Ernest Fleuette

ABSTRACTS OF PAPERS

PRESENTED AT THE WESTERN DIVISION MEETING

DAVIS, CALIFORNIA, JULY 14–16, 1958

A Rapid Method for the Estimation of Serum Proteins in Milk. RUPERT SEALS AND U. S. ASHWORTH, Washington State College, Pullman.

A rapid method for the determination of serum proteins in milk, based on the use of a disulfonic acid dye, orange G, has been developed. The casein is removed by isoelectric precipitation and 15 ml. of the filtrate is placed in a centrifuge tube to which 25 ml. of the dye (0.95 mg/ml) is added. The resulting mixture becomes turbid immediately. The solution is centrifuged and the adsorbance of the supernatant is read in a Beckman Model B Spectrophotometer at 420 mμ. The adsorbance of a standard also is obtained. The difference in adsorbance of the standard and the sample is taken as a measure of the amount of protein.

Effect of Mastitis on Susceptibility of Milk to Lipolysis. N. P. TARASSUK AND M. YAGUCHI, University of California, Davis.

The initial free fatty acid (F.F.A.) titration values (content of free fatty acids in freshly drawn milk) were uniformly greater in mastitic milk than in nonmastitic milk. In severely mastitic milk samples the mean value was about 75% greater. Mastitic milk was found to be considerably and uniformly less susceptible to induced lipolysis (F.F.A. of activated aged milk, minus initial F.F.A.) on activation by shaking than mastitis-negative milk, but the total free fatty acid titration values after activation and aging are about the same for both, since mastitic milk has higher values to begin with. Reduced susceptibility of mastitic milk to induced lipolysis is attributed to the higher content of initial free fatty acids which act to some extent as foam inhibitors.

Effect of Solids Level on Curd Tension. U. S. ASHWORTH AND J. L. NEBE, Washington State College, Pullman.

Using spray-dried nonfat milk solids, produced both in the college pilot plant and commercially, it has been shown that a relationship exists between total solids and curd tension. Samples reconstituted to levels between 9 and 13% give a 20- to 25-g. increase for each 1% increase in solids. The importance of this relationship has been emphasized in the cottage cheese industry, where low heat N.F.D.M.S. may be added to raise and standardize the solids level of skim, giving greater uniformity in quality, higher yields per vat, thus increasing vat capacity, and a shortened process. A higher solids level also counteracts the heat treatment effect upon the skim, given it for better keeping quality.

Propagation of Lactic Starter Cultures in Sterile, Canned Skimmilk. H. K. ZIMMERMAN, R. L. HICKS, L. T. KESTER, AND J. B. MANN, Carnation Research Laboratory, Van Nuys, California.

The propagation of lactic starter cultures in sterile, canned skimmilk has been carried on successfully for a period of more than 2 yr.

In this method, raw skimmilk which has been pretested for the absence of inhibitory material is fortified to 11% total solids with pretested N.F.D.M. and processed under H.T.S.T. conditions.

Prior to inoculation, the can top is flamed for approximately 10 sec. and a hole is punched in the top with a flamed opener of a special design. Inoculation is made in the usual manner. The can top is covered with sterile aluminum foil and the can incubated at 72° F.

The advantages claimed for this method over the conventional method of propagating lactic starters are as follows: *1*) a great savings in time can be realized, since the mother culture medium does not have to be prepared daily, *2*) the culture medium is exactly the same from day to day, resulting in greater uniformity and higher levels of activity in cultures so maintained, and *3*) the use of a sterile, inhibitor-free medium eliminates the possibility of losing cultures due to an inhibitor being present in the culture medium. This method has been well accepted in a number of plants manufacturing cottage cheese and buttermilk.

Influence of Cane Molasses on Feed Flavor in Milk. S. W. MEAD AND W. L. DUNKLEY, University of California, Davis.

Eight cows were selected on the basis of relative freedom of their milk from flavor defects, and divided into two groups. Roughages were withheld from both groups for at least 4 hr. before milking, and ground barley was fed approximately according to production at milking time. One group was kept as control and the other received, in addition to ground barley, 3 lb. of molasses the first day, 2 lb. the second, and 1 lb. the third. A two-day change-over period was allowed, then the groups were interchanged and the procedure repeated. Milk samples were collected and about 30 min. after milking were scored by four judges.

Feeding 1 lb. of cane molasses per cow 0.5–2 hr. before milking caused a slight feed flavor of a character and intensity that would not be objectionable in a commercial milk supply. Two or more pounds of molasses fed under the same conditions caused a distinct feed flavor that should be avoided.

On the basis of these results, the common method of feeding molasses in concentrate mixtures at milking time may be considered entirely acceptable with respect to milk flavor. As much as 10 lb. of a concentrate mixture containing 10% of molasses can be fed at milking time without causing objectionable off-flavor in the milk. However, care should be taken to prevent consumption of two or more pounds of molasses during the critical period (particularly 0.5–2 hr.) before milking.

Prevention of Nutrient Losses in Silage by Use of Plastic Covers.[1] A. S. HODGSON, F. R. MURDOCK, AND J. R. HARRIS, Western Washington Experiment Station, Puyallup.

In 1956, a bunker silo was filled with corn and subjected to two treatments. One end was covered with a polyvinyl chloride plastic sheet and the other end left exposed. Dry matter losses from spoilage were 13.0 and 17.0%, and leaching losses of dry matter, 0.4 and 5.7%, respectively, for covered and uncovered silages. In 1957, the bunker silo was filled with corn and completely covered with plastic strips and weighted with a sawdust layer. Leaching loss was eliminated and 1.17% of the dry matter was lost as spoilage. Fermentation losses accounted for 16.5% of the dry matter.

Nutrient losses were studied in a gas-tight polyvinyl chloride sleeve. In 1956, with corn silage, 92.3% of the dry matter and 98.0% of the crude protein were recovered. In 1957, 92.6% of grass silage dry matter was recovered.

Recovery of dry matter from a rectangular stack of corn silage covered with black polyethylene plastic was 85.2%. Dry matter recovery from a grass silage stack so covered was 70.1%.

Nutrient Losses and Feeding Value of Wilted and Direct-Cut Forages Stored in Tower Silos.[1] F. R. MURDOCK, A. S. HODGSON, AND J. R. HARRIS, Western Washington Experiment Station, Puyallup.

Two lots of grass silage of uniform forage composition were stored in larger tower silos. One lot was harvested by direct-cut methods (average D.M. 21.3%), whereas the other was allowed to partially wilt (average D.M. 29.8%) before ensiling. Dry matter losses during storage were greater for the direct-cut silage. Seepage and fermentation losses were 5.5 and 8.5%, respectively, of the total dry matter ensiled by the direct-cut method, and 1.1 and 6.4%, respectively, by the wilted method. Total dry matter losses were 14.0 and 7.5%, respectively, for direct-cut and wilted silages as determined by input-output measurements, and 13.6 and 7.6%, respectively, as determined by buried bag techniques.

Average dry matter consumption by Holstein heifers fed these silages were 13.6 and 15.2 lb. daily and average weight gains were 0.52 and 0.65 lb. daily for direct-cut and wilted silages, respectively.

Average dry matter digestibilities by Holstein heifers were 52.7% for the direct-cut silage and 56.6% for the wilted silage.

Seasonal Influences on Artificial Breeding with Washington Dairy and Beef Bulls. M. H. EHLERS AND R. E. ERB, Washington State College, Pullman.

This study was based on 3 yr. of data involving 281,308 first services. Sixty-one bulls of the Guernsey, Holstein, Jersey, Hereford, and Milking Shorthorn breeds were used, and over 92% of the services represented bulls in continuous usage for a minimum of 12 mo. Differences between months in 60- to 90-day nonreturns were highly significant, averaging lowest in March (67.5%) and highest in September (72.2%). The months from June through October were above average, and the remaining months were below average, in nonreturns.

Considering all breedings, there were two distinct peaks in numbers seasonally—in June and December. However, there were pronounced breed differences with Herefords and Milking Shorthorn bulls having a single peak in June-July and in Holsteins having a pronounced peak in November-December. Jerseys and Guernseys crested in May-June and December-January. Data were not available on the breed of the cows, but they would be almost exclusively characterized as dairy.

Effects of Progesterone Administration on Reproductive Efficiency. K. R. JOHNSON, Idaho Agricultural Experiment Station, Moscow.

The effect of the administration of progesterone on reproductive efficiency was studied, using 166 apparently normal females. The animals in both the treated and control groups were free from detectable infection, disease, and other reproductive abnormalities. All females in both groups had well-developed Graafian follicles at the time of breeding, as determined by rectal examination. The 82 treated

[1] This study was supported in part by Funds from the Dairy Cattle Research Branch under Cooperative Agreement 12-14-100-769 (52).

animals had a first-service conception of 70.7%; the 84 controls had only 46.4%. The administration of progesterone to apparently normal cows at first breeding increased the breeding efficiency of the treated group 24.3% over the controls for first-service conceptions. The total breeding efficiency for the treated group was 72.6%, 82 conceptions from 113 services compared to 56.4%, 84 conceptions from 149 services for the controls. The additional progesterone may have increased the "uterine milk" by stimulating the endometrium, which may have aided nidation and cut down on the percentage of early embryonic death in the treated group.

Use of Pellets Made from Finely Ground Alfalfa in Dairy Rations. R. G. Fossland and J. B. Fitch, University of Arizona, Tucson.

Two trials involving the use of pelleted, finely ground alfalfa hay were conducted from January to August, 1957. In the first trial, a pellet made of 15% finely ground hay plus 25% grain mixture was compared to baled hay and loose grains in a double-reversal feeding trial. In the second trial, three rations were compared: (*1*) Alfalfa hay and hegari silage, (*2*) Hegari silage plus alfalfa pellets, (*3*) Alfalfa pellets plus straw. A grain mixture was fed along with all three. Eighteen lactating cows, divided into three groups, were used in each trial.

No significant changes were noted in milk production or body weight attributable to any of the rations. Cows fed the complete pelleted ration in the first trial consumed only enough to maintain body weight, plus limited milk production. The percentage of butterfat in the milk from these cows went as low as 1.5%, rumination was poor, chronic bloat was observed, reproductive efficiency was lowered, and a depraved appetite, manifested in fence-chewing, was observed.

In the second trial, there were no differences noted in milk production or body weight, nor were any of the bad effects of Trial One noted. Even small amounts of barley straw satisfied the craving for roughage.

Feeding Fat and Propionate to Dairy Cattle. J. Stein, D. W. Cassard, and M. Wade, University of California, Davis.

Twelve Holstein cows were divided into four comparable groups on the basis of stage of lactation, milk production, and body weight. In a reversal-type feeding experiment each group received the following rations: basal, basal + 2.8% propionate, basal + 7.5% animal fat, and basal + 2.8% propionate + 7.5% animal fat fed at the rate of 1:6. Each treatment period was of 6 wk. duration, with no transition. At the end of each treatment period blood samples were withdrawn at 3-, 6-, and 8-hr. intervals after feeding, for the following determinations: plasma lipids, glucose, ketones, vitamin A, and carotene. Three daily consecutive body weights were taken. In addition, feed consumption and milk production were recorded daily for each treatment studied. These results will be discussed.

Occurrence of Post-Conception Estrus in Seven Herds of Jersey Cattle. D. F. Rahlmann and S. W. Mead, University of California, Davis.

Breeding records from seven cooperator herds in the University of California loan bull project were used in this study. Only cows with at least one normal calving during their reproductive life were considered. Estrus at varying intervals following conception was observed in 4.46% of 3,159 cows during the period from 1935 to 1957. The occurrence of estrus ranged from 11 to 213 days after conception, with a mean of 63.0 ± 3.97 days. Post-conception estrus occurred almost twice as frequently during the first gestation as in any subsequent period. Equal numbers of female and male fetuses were involved in gestations where estrus after conception was observed. Seasonal variations ranged from a low of five in April to a high of 18 in July.

An Enzyme of Colostrum Capable of Hydrolyzing Acetylcholine. J. E. Montoure, T. L. Forster, and H. A. Bendixen, Washington State College, Pullman.

Colostrum milk has been found to contain an enzyme capable of hydrolyzing acetylcholine. This enzyme is inhibited by low concentrations of eserine sulfate. The amount of this enzyme decreases rapidly as the colostrum composition approaches that of normal milk. In all probability, the enzyme is a cholinesterase.

Effect of Feeding Stilbestrol to Holstein Steers. R. O. Rupp, G. M. Ward, and M. H. Hazaleus, Colorado Agricultural Experiment Station, Fort Collins.

Sixteen Holstein steers averaging 372 lb. were divided into two groups and were fed alfalfa hay, corn silage, and 4–12 lb. of concentrate. One group of eight steers received diethylstilbestrol premix to supply 10 mg. per head per day. The other eight steers served as controls. The animals were slaughtered after 278 days on feed. The stilbestrol-fed group and control group, respectively, averaged 1,032 and 968 lb. at slaughter; had daily gains of 2.39 and 2.13 lb.; dressing percentage of 53.6 and 54.4%; and a return above feed cost of $35.70 and $22.91. All of the animals in both groups yielded carcasses grading U.S. Standard. There was no difference in percentages of the various wholesale cuts for the two groups. No undesirable side-effects from stilbestrol feeding were noted.

Effects of Tranquilizing Drugs on Milk Production and Fat Percentage of Milk. D. C. JORDAN AND G. M. WARD, Colorado State University, Fort Collins.

The purpose of this study was to determine the effect of an injectable tranquilizing drug[2] on the behavior of nervous cows and its effect on milk and fat production. Intramuscular and intravenous injections of 0.5 to 1.0 mg/kg of body weight were administered to first-calf heifers for a period of several days postpartum and daily to four mature, high-producing Holstein cows for two periods of six days each. Muscular injections of the higher levels resulted in depressed activity and appetite during the entire course of treatment. Intravenous injections produced sedation in 5 min. which disappeared in from 4 to 5 hr. Milk production did not appear ot be affected and the fat increased, although probably not significantly. Nervous first-calf heifers responded well to the milking routine after several treatments.

A Comparison of Two Levels of Grain Feeding of Dairy Cattle on Pasture. E. P. SMITH, Montana State College, Bozeman.

An 84-day feeding trial was conducted to compare two levels of grain feeding to lactating cows on lush pasture. The cows of Group I received 2 lb. of grain each day, regardless of production. The cows of Group II were fed grain at a rate of 1 lb. of grain daily for every 3 lb. of fat-corrected milk produced above 20 lb. Other than grain feeding, the two groups of cows were managed exactly the same.

The total amount of F.C.M. produced by Groups I and II during this experiment was 38,374 and 37,877 lb., respectively. Both groups of cows gained weight during the experiment. The cows of Group I consumed 1,794 more pounds of TDN from pasture than the cows of Group II.

Effect of Feeding Different Sources of Carotene and/or Vitamin A on Bovine Blood, Liver, and Milk Fat. D. D. BULLIS, P. H. WESWIG, I. R. JONES, AND J. F. BONE, Oregon State College, Corvallis.

The sources of supplementation used in this study included carrot oil, alfalfa leaf meal, synthetic beta-carotene, and a synthetic vitamin A fed at 65, 130, and 390 μg. of beta-carotene per kilogram of body weight. The carotene and vitamin A contents of the blood plasma reflect the higher levels of supplementation regardless of source, with the exception of a rather constant plasma carotene level when synthetic vitamin A was fed. Liver carotene and vitamin A increased with the level fed and varied significantly with the source. The highest liver carotene values, averaging 5.9 γ/g, were obtained from cows fed alfalfa leaf meal at the highest level; whereas, the highest level of synthetic vitamin A produced the highest vitamin A liver values, averaging 124.4 γ/g. The carotene and vitamin A level of milk fat increased significantly with the increase in level fed, with alfalfa leaf meal having the highest carotene values, averaging 3.5 γ/g at the 390 level, and with synthetic vitamin A at the highest level producing milk fat with the highest vitamin A values, averaging 8.5 γ/g.

Significant correlations were found as follows:

Blood plasma carotene and plasma vitamin A	.63[3]
Blood plasma carotene and liver carotene	.84[4]
Blood plasma carotene and milkmlik carotene	.72[3]
Blood plasma vitamin A and liver carotene	.63[3]
Blood plasma vitamin A and milk vitamin A	.62[3]
Liver carotene and milk carotene	.94[4]
Liver vitamin A and milk vitamin A	.79[4]

Oral Administration of Penicillin as a Bloat Preventive. K. R. JOHNSON AND J. W. BAILEY, Idaho Agricultural Experiment Station, Moscow.

Forty-two lactating dairy animals were divided into two groups at random. The susceptibility of the animals to bloat was not known. The treated group was given oral administrations of 62,500 units (38 mg.) of penicillin daily. The antibiotic was given with the grain ration each morning, beginning 14 days before the cows were pastured until the end of the experiment. The animals were given a severe challenge of lush alfalfa pasture for 55 days. Eight of the 23 treated cows (34.7%) bloated a total of 15 times, with a range of one to three times. The remaining 15 treated cows did not bloat. Eleven of the 19 control cows (56.3%) bloated a total of 28 times, with a range of one to four times. Eight of the control cows did not bloat. The difference in the incidence of bloat between the treated and control groups was significant at the 0.01 level of probability. There was no difference in the severity of bloat in the treated and untreated animals. Two chronic-bloat cows were changed from the control to the treated group. Both cows continued to bloat after treatment. On the basis of this limited experiment, it appears that oral administration of penicillin may aid in reducing the incidence, but not the severity, of bloat in cattle.

[2] The tranquilizing drug, Vetame, was supplied by E. R. Squibb and Company through the courtesy of Dr. A. D. Rankin.

[3] Significant at the 5% level.

[4] Significant at the 1% level.

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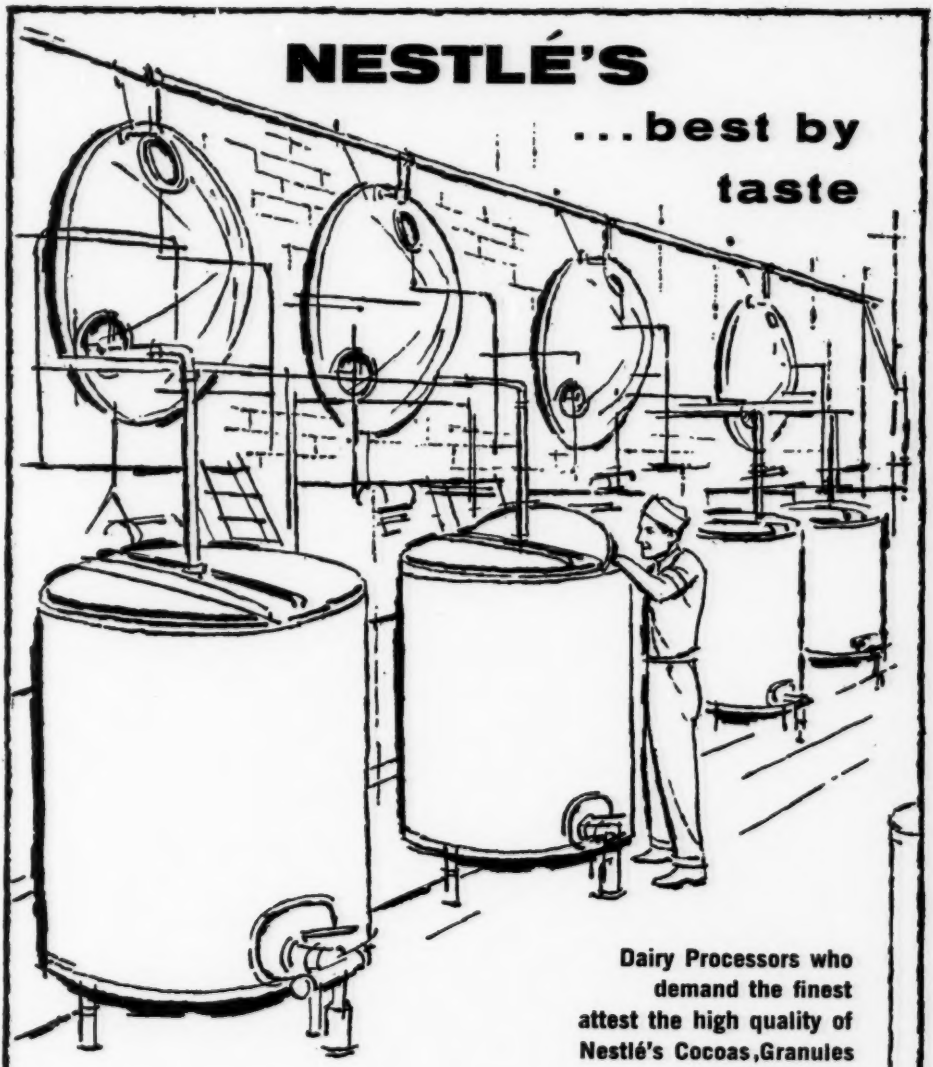

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